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(54) Title: PROTEASE INHIBITORS

(57) Abstract

The present invention provides diacyl carbohydrazide compounds, and pharmaceutically acceptable salts, hydrates and solvates thereof, which inhibit proteases, including cathepsin K, pharmaceutical compositions of such compounds, novel intermediates of such compounds, and methods for treating diseases of excessive bone loss or cartilage or matrix degradation, including osteoporosis; gingival disease including gingivitis and periodontitis; arthritis, more specifically, osteoarthritis and rheumatoid arthritis; Paget's disease; hypercalcemia of malignancy; and metabolic bone disease, comprising inhibiting said bone loss or excessive cartilage or matrix degradation by administering to a patient in need thereof a compound of the present invention.

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PROTEASE INHIBITORS

FIELD OF THE INVENTION

This invention relates in general to diacyl carbohydrazide protease inhibitors, particularly such inhibitors of cysteine and serine proteases, more particularly compounds which inhibit cysteine proteases, even more particularly compounds which inhibit cysteine proteases of the papain superfamily, yet more particularly compounds which inhibit cysteine proteases of the cathepsin family, most particularly compounds which inhibit cathepsin K. Such compounds are particularly useful for treating diseases in which cysteine proteases are implicated, especially diseases of excessive bone or cartilage loss, e.g., osteoporosis, periodontitis, and arthritis.

BACKGROUND OF THE INVENTION

Cathepsins are a family of enzymes which are part of the papain superfamily of cysteine proteases. Cathepsins B, H, L, N and S have been described in the literature. Recently, cathepsin K polypeptide and the cDNA encoding such polypeptide were disclosed in U.S. Patent No. 5,501,969 (called cathepsin O therein). Cathepsin K has been recently expressed, purified, and characterized. Bossard, M. J., et al., (1996) *J. Biol. Chem.* **271**, 12517-12524; Drake, F.H., et al., (1996) *J. Biol. Chem.* **271**, 12511-12516; Bromme, D., et al., (1996) *J. Biol. Chem.* **271**, 2126-2132.

Cathepsin K has been variously denoted as cathepsin O or cathepsin O2 in the literature. The designation cathepsin K is considered to be the more appropriate one.

Cathepsins function in the normal physiological process of protein degradation in animals, including humans, e.g., in the degradation of connective tissue. However, elevated levels of these enzymes in the body can result in pathological conditions leading to disease. Thus, cathepsins have been implicated as causative agents in various disease states, including but not limited to, infections by pneumocystis carinii, trypsanoma cruzi, trypsanoma brucei brucei, and Crithidia fusiculata; as well as in schistosomiasis, malaria, tumor metastasis, metachromatic leukodystrophy, muscular dystrophy, amytrophy, and the like. *See* International Publication Number WO 94/04172, published on March 3, 1994, and references cited therein. *See also* European Patent Application EP 0 603 873 A1, and references cited therein. Two bacterial cysteine proteases from P. gingivallis, called

gingipains, have been implicated in the pathogenesis of gingivitis. Potempa, J., et al. (1994) *Perspectives in Drug Discovery and Design*, **2**, 445-458.

Cathepsin K is believed to play a causative role in diseases of excessive bone or cartilage loss. Bone is composed of a protein matrix in which spindle- or plate-shaped crystals of hydroxyapatite are incorporated. Type I collagen represents the major structural protein of bone comprising approximately 90% of the protein matrix. The remaining 10% of matrix is composed of a number of non-collagenous proteins, including osteocalcin, proteoglycans, osteopontin, osteonectin, thrombospondin, fibronectin, and bone sialoprotein. Skeletal bone undergoes remodelling at discrete foci throughout life. These foci, or remodelling units, undergo a cycle consisting of a bone resorption phase followed by a phase of bone replacement.

Bone resorption is carried out by osteoclasts, which are multinuclear cells of hematopoietic lineage. The osteoclasts adhere to the bone surface and form a tight sealing zone, followed by extensive membrane ruffling on their apical (i.e., resorbing) surface. This creates an enclosed extracellular compartment on the bone surface that is acidified by proton pumps in the ruffled membrane, and into which the osteoclast secretes proteolytic enzymes. The low pH of the compartment dissolves hydroxyapatite crystals at the bone surface, while the proteolytic enzymes digest the protein matrix. In this way, a resorption lacuna, or pit, is formed. At the end of this phase of the cycle, osteoblasts lay down a new protein matrix that is subsequently mineralized. In several disease states, such as osteoporosis and Paget's disease, the normal balance between bone resorption and formation is disrupted, and there is a net loss of bone at each cycle. Ultimately, this leads to weakening of the bone and may result in increased fracture risk with minimal trauma.

Several published studies have demonstrated that inhibitors of cysteine proteases are effective at inhibiting osteoclast-mediated bone resorption, and indicate an essential role for a cysteine proteases in bone resorption. For example, Delaisse, *et al.*, *Biochem. J.*, **1980**, *192*, 365, disclose a series of protease inhibitors in a mouse bone organ culture system and suggest that inhibitors of cysteine proteases (e.g., leupeptin, Z-Phe-Ala-CHN_2) prevent bone resorption, while serine protease inhibitors were ineffective. Delaisse, *et al.*, *Biochem. Biophys. Res. Commun.*, **1984**, *125*, 441, disclose that E-64 and leupeptin are also effective at preventing bone resorption *in vivo*, as measured by acute changes in serum calcium in rats on calcium deficient diets. Lerner, *et al.*, *J. Bone Min. Res.*, **1992**, *7*, 433, disclose that cystatin, an endogenous cysteine protease inhibitor, inhibits PTH stimulated

bone resorption in mouse calvariae. Other studies, such as by Delaisse, *et al.*, *Bone*, **1987**, *8*, 305, Hill, *et al.*, *J. Cell. Biochem.*, **1994**, *56*, 118, and Everts, *et al.*, *J. Cell. Physiol.*, **1992**, *150*, 221, also report a correlation between inhibition of cysteine protease activity and bone resorption. Tezuka, *et al.*, *J. Biol. Chem.*, **1994**, *269*, 1106, Inaoka, *et al.*, *Biochem. Biophys. Res. Commun.*, **1995**, *206*, 89 and Shi, *et al.*, *FEBS Lett.*, **1995**, *357*, 129 disclose that under normal conditions cathepsin K, a cysteine protease, is abundantly expressed in osteoclasts and may be the major cysteine protease present in these cells.

The abundant selective expression of cathepsin K in osteoclasts strongly suggests that this enzyme is essential for bone resorption. Thus, selective inhibition of cathepsin K may provide an effective treatment for diseases of excessive bone loss, including, but not limited to, osteoporosis, gingival diseases such as gingivitis and periodontitis, Paget's disease, hypercalcemia of malignancy, and metabolic bone disease. Cathepsin K levels have also been demonstrated to be elevated in chondroclasts of osteoarthritic synovium. Thus, selective inhibition of cathepsin K may also be useful for treating diseases of excessive cartilage or matrix degradation, including, but not limited to, osteoarthritis and rheumatoid arthritis. Metastatic neoplastic cells also typically express high levels of proteolytic enzymes that degrade the surrounding matrix. Thus, selective inhibition of cathepsin K may also be useful for treating certain neoplastic diseases.

Several cysteine protease inhibitors are known. Palmer, (1995) *J. Med. Chem.*, *38*, 3193, disclose certain vinyl sulfones which irreversibly inhibit cysteine proteases, such as the cathepsins B, L, S, O2 and cruzain. Other classes of compounds, such as aldehydes, nitriles, a-ketocarbonyl compounds, halomethyl ketones, diazomethyl ketones, (acyloxy)methyl ketones, ketomethylsulfonium salts and epoxy succinyl compounds have also been reported to inhibit cysteine proteases. *See* Palmer, *id*, and references cited therein.

U.S. Patent No. 4,518,528 discloses peptidyl fluoromethyl ketones as irreversible inhibitors of cysteine protease. Published International Patent Application No. WO 94/04172, and European Patent Application Nos. EP 0 525 420 A1, EP 0 603 873 A1, and EP 0 611 756 A2 describe alkoxymethyl and mercaptomethyl ketones which inhibit the cysteine proteases cathepsins B, H and L. International Patent Application No. PCT/US94/08868 and European Patent Application No. EP 0 623 592 A1 describe alkoxymethyl and mercaptomethyl ketones which inhibit the cysteine protease IL-1b convertase. Alkoxymethyl and mercaptomethyl ketones have also been described as

inhibitors of the serine protease kininogenase (International Patent Application No. PCT/GB91/01479).

Azapeptides which are designed to deliver the azaamino acid to the active site of serine proteases, and which possess a good leaving group, are disclosed by Elmore *et al.*, *Biochem. J.*, **1968**, *107*, 103, Garker *et al.*, *Biochem. J.*, **1974**, *139*, 555, Gray *et al.*, *Tetrahedron*, **1977**, *33*, 837, Gupton *et al.*, *J. Biol. Chem.*, **1984**, *259*, 4279, Powers *et al.*, *J. Biol. Chem.*, **1984**, *259*, 4288, and are known to inhibit serine proteases. In addition, Magrath *et al.*, *J. Med. Chem.*, **1992**, *35*, 4279, Baggio *et al.*, Biochemistry, **1996**, *35*, 3551 and Xing *et al.*, *J. Med. Chem.* **1998**, 41, 1344 discloses certain azapeptide esters as cysteine protease inhibitors.

Diacyl carbohydrazides have recently been disclosed as inhibitors of cathepsin K by Thompson *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, **1997**, *94*, 14249 and in International Patent Application No. WO 97/16433.

Antipain and leupeptin are described as reversible inhibitors of cysteine protease in McConnell et al., *J. Med. Chem.*, *33*, 86; and also have been disclosed as inhibitors of serine protease in Umezawa et al., 45 *Meth. Enzymol.* 678. E64 and its synthetic analogs are also well-known cysteine protease inhibitors (Barrett, *Biochem. J.*, *201*, 189, and Grinde, *Biochem. Biophys. Acta*, , *701*, 328).

Thus, a structurally diverse variety of cysteine protease inhibitors have been identified. However, these known inhibitors are not considered suitable for use as therapeutic agents in animals, especially humans, because they suffer from various shortcomings. These shortcomings include lack of selectivity, cytotoxicity, poor solubility, and overly rapid plasma clearance. A need therefore exists for methods of treating diseases caused by pathological levels of cysteine proteases, including cathepsins, especially cathepsin K, and for novel inhibitor compounds useful in such methods.

We have now discovered a novel class of diacyl carbohydrazide compounds which are protease inhibitors, most particularly of cathepsin K.

SUMMARY OF THE INVENTION

An object of the present invention is to provide diacyl carbohydrazide protease inhibitors, particularly such inhibitors of cysteine and serine proteases, more particularly such compounds which inhibit cysteine proteases, even more particularly such compounds which inhibit cysteine proteases of the papain superfamily, yet more particularly such

compounds which inhibit cysteine proteases of the cathepsin family, most particularly such compounds which inhibit cathepsin K, and which are useful for treating diseases which may be therapeutically modified by altering the activity of such proteases.

Accordingly, in the first aspect, this invention provides a compound according to Formula I.

In another aspect, this invention provides a pharmaceutical composition comprising a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient.

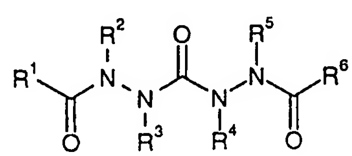
In yet another aspect, this invention provides intermediates useful in the preparation of the compounds of Formula I.

In still another aspect, this invention provides a method of treating diseases in which the disease pathology may be therapeutically modified by inhibiting proteases, particularly cysteine and serine proteases, more particularly cysteine proteases, even more particularly cysteine proteases of the papain superfamily, yet more particularly cysteine proteases of the cathepsin family, most particularly cathepsin K.

In a particular aspect, the compounds of this invention are especially useful for treating diseases characterized by bone loss, such as osteoporosis and gingival diseases, such as gingivitis and periodontitis, or by excessive cartilage or matrix degradation, such as osteoarthritis and rheumatoid arthritis.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compounds of Formula I:



I

R^1 and R^6 are independently selected from the group consisting of C_{5-6}alkyl; C_{2-6}alkenyl; C_{3-11}cycloalkyl-C_{0-6}-alkyl; Ar-C_{0-6}alkyl; Het-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; Ar-C_{2-6}alkynyl; $CH(R^7)Ar$; $CH(R^7)OAr$; NR^7R^8; and $CH(R^7)NR^8R^9$;

R', R^2, R^3, R^4, R^5, R^8, R^{10}, R^{11}, and R^{14} are independently selected from the group consisting of H; C_{1-6}alkyl; C_{2-6}alkenyl; Ar-C_{0-6}alkyl; Het-C_{0-6}alkyl; and C_{3-11}cycloalkyl-C_{0-6}alkyl;

R^7 and R^{13} are independently selected from the group consisting of H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; C_{3-11}cycloalkyl-C_{0-6}-alkyl; Ar-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Ar-C_{2-6}alkynyl; Het-C_{0-6}alkyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; C_{1-6}alkyl, which may optionally be substituted by OR^{10}, SR^{10}, and $NR^{10}R^{11}$; N(R')CO_2R'; CO_2R'; $CONR^{10}R^{11}$; and N(C=NH)NH_2;

R^7 and R^8 may optionally be combined to form a pyrrolidine or piperidine ring;

R^{10} and R^{11} may optionally be combined to form a pyrrolidine, piperidine, or morpholine ring;

R^9 is H; R^{12}; $R^{12}C(O)$; $R^{12}C(S)$; $R^{12}OC(O)$; $R^{12}OC(O)NR^{11}CH(R^{13})C(O)$; $R^{12}SO_2$; $R^{12}SO_2NR^{11}CH(R^{13})C(O)$; R^{12}R'NC(O); R^{12}R'NCS; or $COCH(R^{13})NR^{14}R^{15}$;

R^{12} is C_{1-6}alkyl, which may be optionally substituted by $NR^{10}R^{11}$, C_{2-6}alkenyl, or C_{2-6}alkynyl; Ar-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Ar-C_{2-6}alkynyl; Het-C_{0-6}alkyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; C_{3-11}cycloalkyl, which may be optionally substituted with C_{1-6}alkyl, $(CH_2)_{1-6}CO_2$R', or adamantyl;

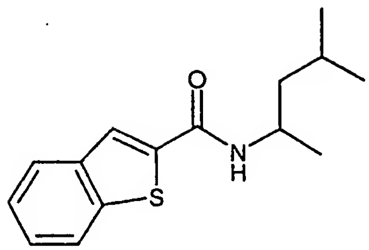
R^{15} is R^{12}; $R^{12}C(O)$; $R^{12}C(S)$; $R^{12}OC(O)$; $R^{12}OC(O)NR^{11}CH(R^{13})C(O)$; $R^{12}SO_2$,; $R^{12}SO_2NR^{11}CH(R^{13})C(O)$; R^{12}R'NC(O); or R^{12}R'NCS;

and pharmaceutically acceptable salts, hydrates and solvates thereof.

Compounds of Formula I wherein R^2, R^3, R^4 or R^5 is H are preferred.

Also preferred are compounds of Formula I wherein:

R^1 is



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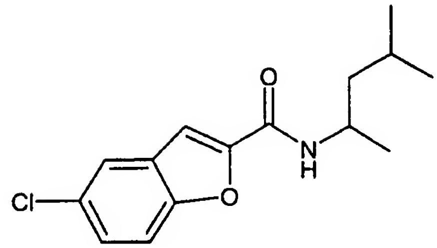

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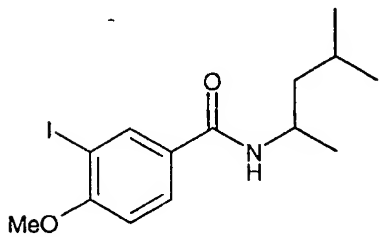

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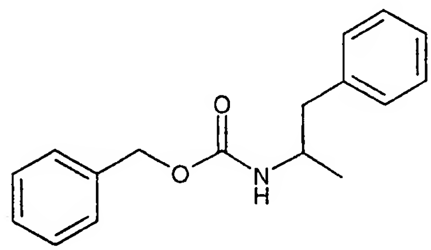

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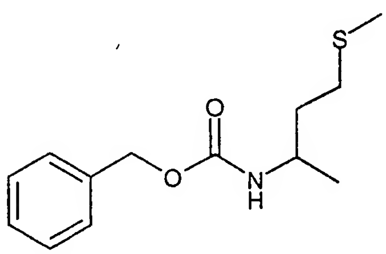

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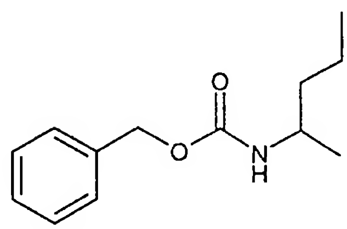

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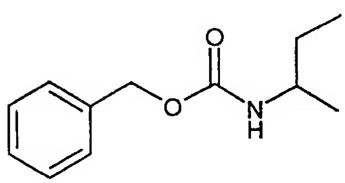

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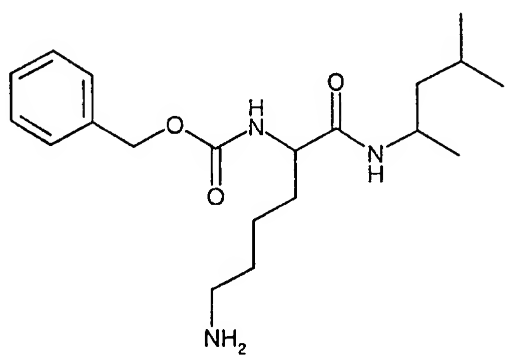

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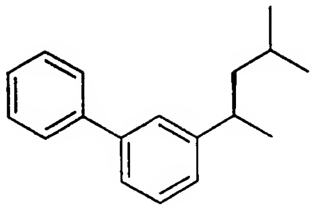
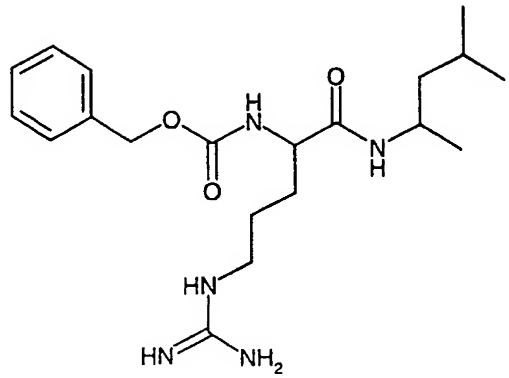
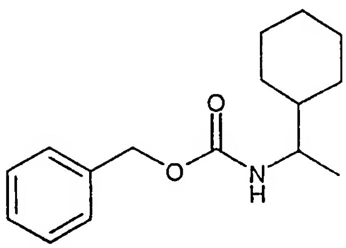
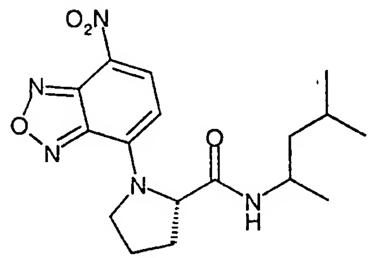
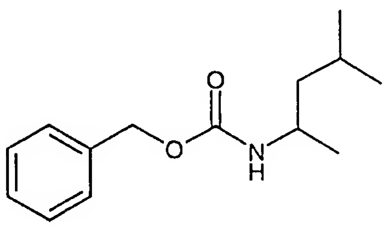
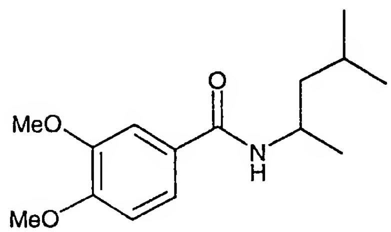
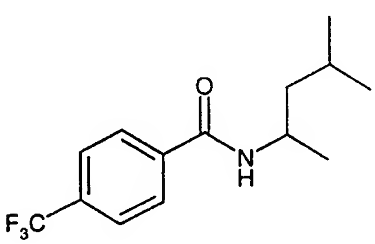
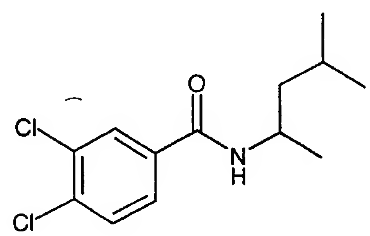
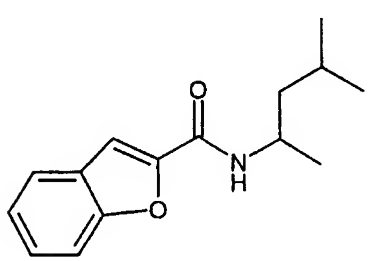
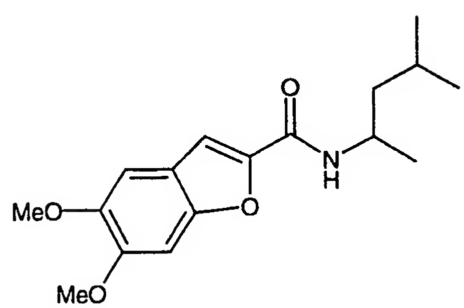
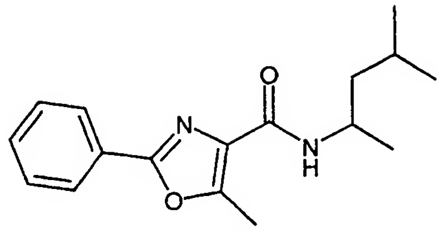
;
;

;
;

;
;

;
;

;
;



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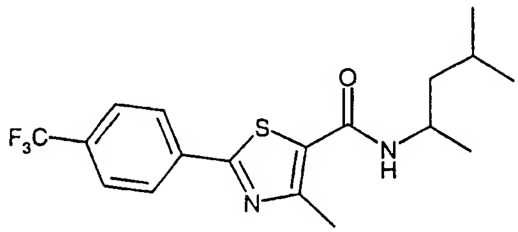

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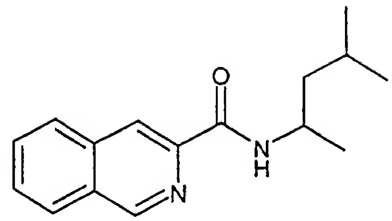

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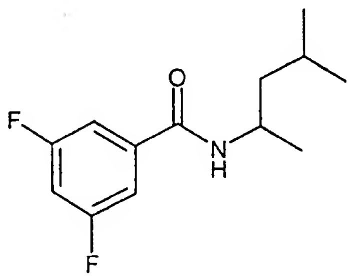

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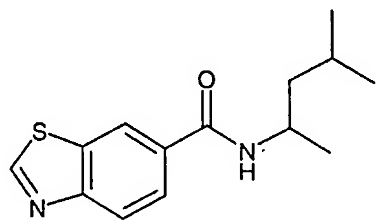

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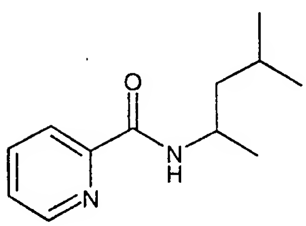

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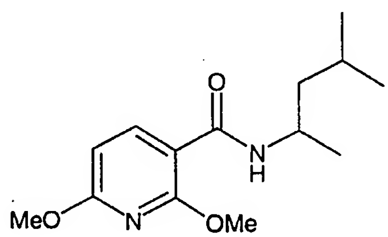

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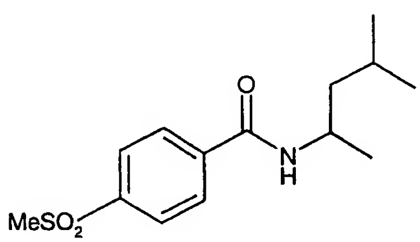

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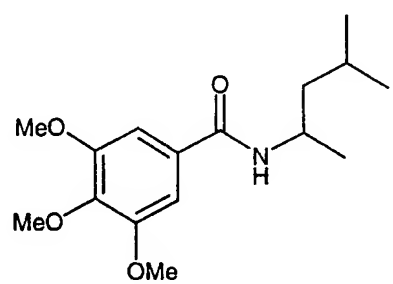

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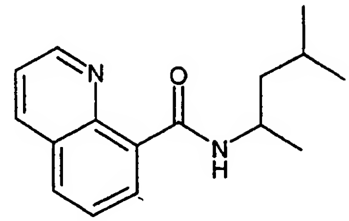
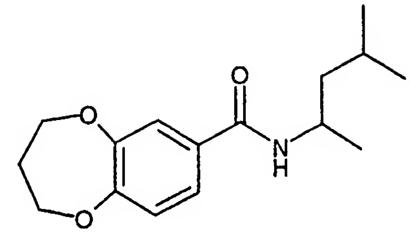
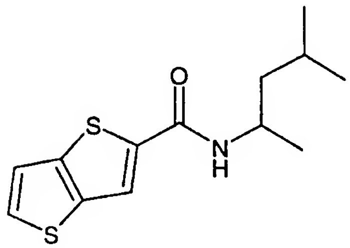
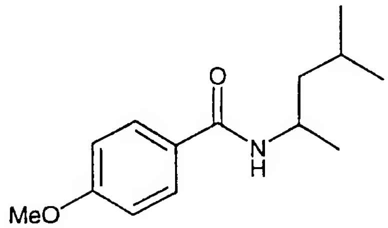
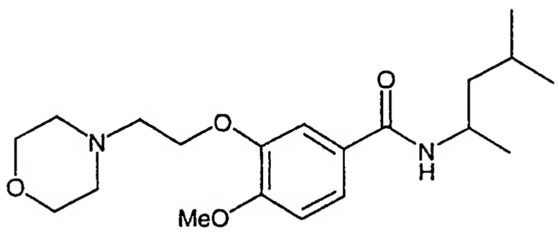
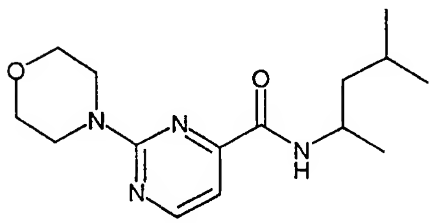
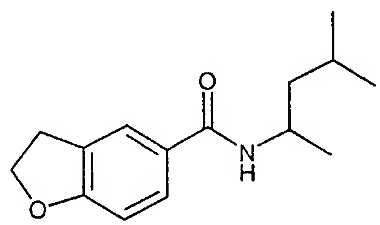
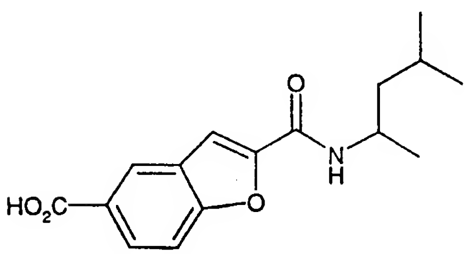
 ;  ;

 ;  ;

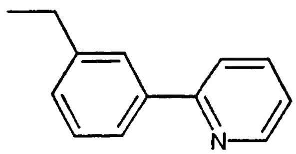
 ;

 ;  ;

 ;

;and

R^6 is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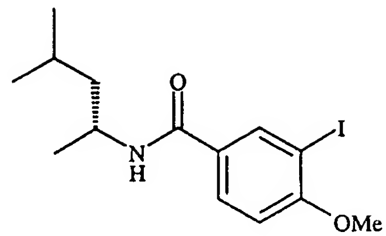
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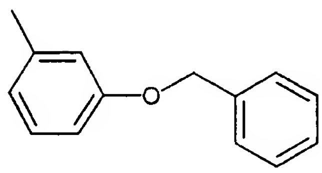
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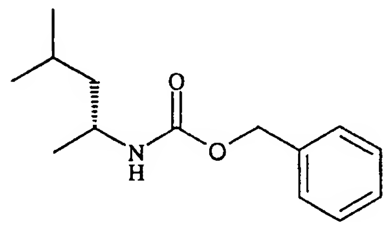
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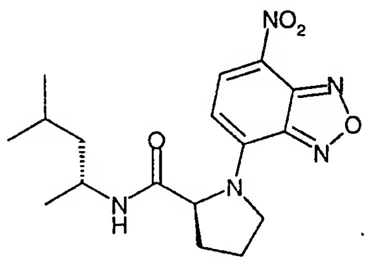
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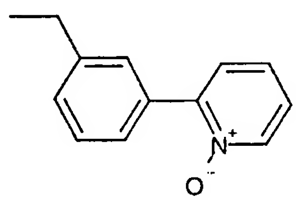


Compounds of Formula I selected from the following group are more preferred embodiments of the present invention:

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-valinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-phenylalanyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-isoleucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-methionyl)]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide;

(2S)-2-[N-(N-benzyloxycarbonyl-2-aminobutyryl)]-2'-[N'-(N-benzyloxycarbonyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide;

(2R)-2-[N-(3-benzyloxybenzoyl)]-2'-[N'-[4-methyl-2-(3-phenylphenyl)pentanoyl]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-arginyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylglycinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylalanyl)]carbohydrazide;

2,2'-[N,N'-bis[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3,4-dimethoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(4-trifluoromethylbenzoyl)-L-leucinyl]]carbohydrazide;

2-[N-[N-(3,4-dichlorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzofuran-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5,6-dimethoxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-methyl-2-phenyloxazol-4-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1'-N'-methyl-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3-isoquinolinoyl)-L-leucinyl]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-chlorobenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3,5-difluorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzothiazol-6-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1-(N-methyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-picolinoyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(2,6-dimethoxynicotinoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(4-methanesulfonylbenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(3,4,5-trimethoxybenzoyl)-L-leucinyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(8-quinolinoyl)-L-leucinyl]]carbohydrazide;

2-[N-[N-[3,4-(1,3-propylenedioxy)benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-(N-thieno[2,3-b]thiophen-2-ylcarbonyl-L-leucinyl)]carbohydrazide;

2-[N-[N-(4-methoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[4-methoxy-3-[2-(4-morpholino)ethoxy]benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[2-(4-morpholino)pyrimidin-4-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(2,3-dihydrobenzofuran-5-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-carboxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)-2'-[N'-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide;

2-[N-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]-2-[N-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;
2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide; and
2,2'-[N,N'-bis[N-(3-iodo-4-methxoy)-L-leucinyl]]carbohydrazide.

Compounds of Formula I selected from the following group are particularly preferred embodiments of the present invention:
2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;
2-[N-[N-(5-chlorobenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide; and
2,2'-[N,N'-bis[N-(3-iodo-4-methxoy)-L-leucinyl]]carbohydrazide.

Definitions

The present invention includes all hydrates, solvates, complexes and prodrugs of the compounds of this invention. Prodrugs are any covalently bonded compounds which release the active parent drug according to Formula I *in vivo*. If a chiral center or another form of an isomeric center is present in a compound of the present invention, all forms of such isomer or isomers, including enantiomers and diastereomers, are intended to be covered herein. Inventive compounds containing a chiral center may be used as a racemic mixture, an enantiomerically enriched mixture, or the racemic mixture may be separated using well-known techniques and an individual enantiomer may be used alone. In cases in which compounds have unsaturated carbon-carbon double bonds, both the cis (Z) and trans (E) isomers are within the scope of this invention. In cases wherein compounds may exist in tautomeric forms, such as keto-enol tautomers, each tautomeric form is contemplated as being included within this invention whether existing in equilibrium or predominantly in one form.

The meaning of any substituent at any one occurrence in Formula I or any subformula thereof is independent of its meaning, or any other substituent's meaning, at any other occurrence, unless specified otherwise.

Abbreviations and symbols commonly used in the peptide and chemical arts are used herein to describe the compounds of the present invention. In general, the amino acid

abbreviations follow the IUPAC-IUB Joint Commission on Biochemical Nomenclature as described in *Eur. J. Biochem.*, 158, 9 (1984).

The term "amino acid" as used herein refers to the D- or L- isomers of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine.

"C_{1-6}alkyl" as applied herein is meant to include substituted and unsubstituted methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl and t-butyl, pentyl, n-pentyl, isopentyl, neopentyl and hexyl and the simple aliphatic isomers thereof. Any C_{1-6}alkyl group may be optionally substituted independently by one to five halogens, S R^{16}, O R^{16}, $N(R^{16})_2$, $C(O)N(R^{16})_2$, carbamyl or C_{1-4}alkyl, where R^{16} is C_{1-6}alkyl. C_0alkyl means that no alkyl group is present in the moiety. Thus, Ar-C_0alkyl is equivalent to Ar.

"C_{3-11}cycloalkyl" as applied herein is meant to include substituted and unsubstituted cyclopropane, cyclobutane, cyclopentane, cyclohexane, cycloheptane, cyclooctane, cyclononane, cyclodecane, cycloundecane.

"C_{2-6} alkenyl" as applied herein means an alkyl group of 2 to 6 carbons wherein a carbon-carbon single bond is replaced by a carbon-carbon double bond. C_{2-6}alkenyl includes ethylene, 1-propene, 2-propene, 1-butene, 2-butene, isobutene and the several isomeric pentenes and hexenes. Both cis and trans isomers are included.

"C_{2-6}alkynyl" means an alkyl group of 2 to 6 carbons wherein one carbon-carbon single bond is replaced by a carbon-carbon triple bond. C_{2-6} alkynyl includes acetylene, 1-propyne, 2-propyne, 1-butyne, 2-butyne, 3-butyne and the simple isomers of pentyne and hexyne.

"Halogen" means F, Cl, Br, and I.

Ar is phenyl; naphthyl, optionally substituted by one or more of Ph-C_{0-6}alkyl, Het-C_{0-6} alkyl, C_{1-6}alkyl, C_{1-6}alkoxy, Ph-C_{0-6}alkoxy, Het-C_{0-6}alkoxy, OH, $(CH_2)_{1-6}NR^{10}R^{11}$, $O(CH_2)_{1-6}NR^{10}R^{11}$, $(CH_2)_{0-6}CO_2R'$, $O(CH_2)_{1-6}CO_2R'$, SR^{10}, SO_2R^{10}, CF_3, nitro or halogen; Ph may be optionally substituted with one or more of C_{1-6}alkyl, C_{1-6}alkoxy, OH, $(CH_2)_{1-6}NR^{10}R^{11}$, $O(CH_2)_{1-6}NR^{10}R^{11}$, $(CH_2)_{0-6}CO_2R'$, $O(CH_2)_{1-6}CO_2R'$, SR^{10}, SO_2R^{10}, CF_3, cyano or halogen; two C_{1-6}alkyl or C_{1-6}alkoxy groups may be combined to form a 5-7 membered ring, saturated or unsaturated, fused onto the Ar ring;

As used herein "Het" or "heterocyclic" represents a stable 5- to 7-membered monocyclic, a stable 7- to 10-membered bicyclic, or a stable 11- to 18-membered tricyclic

heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to three heteroatoms selected from the group consisting of N, O and S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized, and including any bicyclic group in which any of the above-defined heterocyclic rings is fused to a benzene ring. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure, and may be optionally substituted as with Ar (including on the nitrogens) Examples of such heterocycles include piperidinyl, piperazinyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolodinyl, 2-oxoazepinyl, azepinyl, pyrrolyl, 4-piperidonyl, pyrrolidinyl, pyrazolyl, pyrazolidinyl, imidazolyl, pyridyl, pyrazinyl, oxazolidinyl, oxazolinyl, oxazolyl, isoxazolyl, morpholinyl, thiazolidinyl, thiazolinyl, thiazolyl, quinuclidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, benzoxazolyl, furyl, pyranyl, tetrahydrofuryl, tetrahydropyranyl, thienyl, benzoxazolyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl.

"5-7 membered ring, saturated or unsaturated, fused onto the Ar ring" means a fused bicyclic ring system such as indane, 1,2,3,4-tetrahydrodecalin, methylenedioxyphenyl, 1,2-ethylenedioxyphenyl and 1,3-propylenedioxyphenyl.

Here and throughout this application the term C_0 denotes the absence of the substituent group immediately following; for instance, in the moiety ArC_{0-6}alkyl, when C is 0, the substituent is Ar, e.g., phenyl. Conversely, when the moiety ArC_{0-6}alkyl is identified as a specific aromatic group, e.g., phenyl, it is understood that C is 0.

Certain radical groups are abbreviated herein. t-Bu refers to the tertiary butyl radical, Boc refers to the t-butyloxycarbonyl radical, Fmoc refers to the fluorenylmethoxycarbonyl radical, Ph refers to the phenyl radical, Cbz refers to the benzyloxycarbonyl radical.

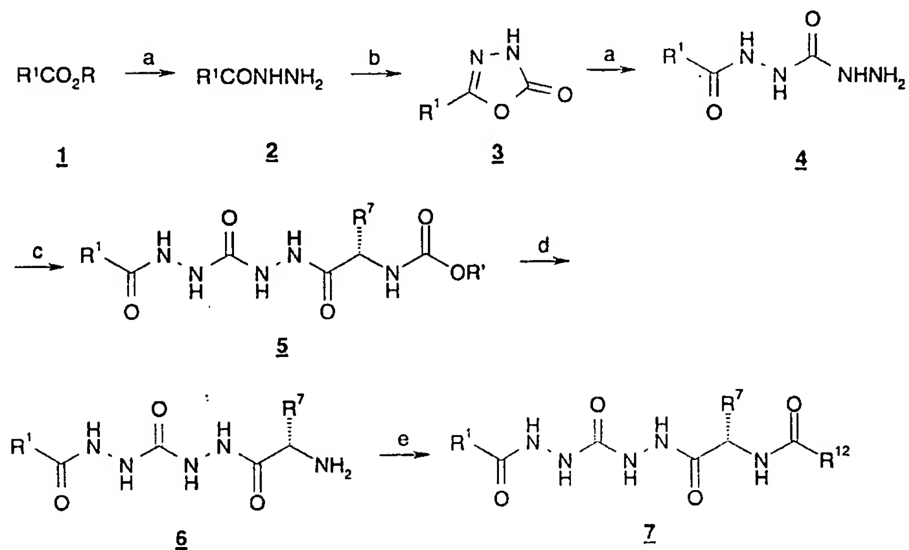
Certain reagents are abbreviated herein. DCC refers to dicyclohexylcarbodiimide, DMAP is 2,6-dimethylaminopyridine, EDC refers to N-ethyl-N'(dimethylaminopropyl)-carbodiimide. HOBT refers to 1-hydroxybenzotriazole, DMF refers to dimethyl formamide, BOP refers to benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate, DMAP is dimethylaminopyridine, NMM is N-methylmorpholine, TFA refers to trifluoroacetic acid, THF refers to tetrahydrofuran. Jones reagent is a solution of chromium trioxide, water, and sulfuric acid well-known in the art.

Methods of Preparation

The compounds of the present invention may be conveniently prepared by the methods set forth in Schemes 1 - 3 below.

Compounds of the Formula I, wherein $R^1 \neq R^6$ and R^2, R^3, R^4 and R^5 are H, are prepared by methods analogous to those described in Scheme 1.

Scheme 1

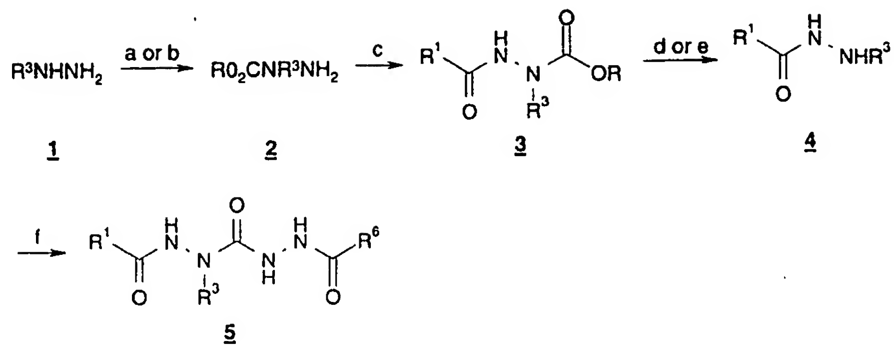


a) $H_2NNH_2 \cdot H_2O$, MeOH; b) Cl_2CO, PhMe; c) $RO_2CNHCH(R^7)CO_2H$, EDC·HCl, 1-HOBT, DMF; d) (R = *t*-Bu), TFA, CH_2Cl_2; (R = Bn), HBr, HOAc; e) $R^{12}CO_2H$, EDC·HCl, 1-HOBT, DMF.

Treatment of 1-Scheme 1 with hydrazine hydrate in a protic solvent (such as methanol or ethanol) provided 2-Scheme 1, which was treated phosgene in toluene to afford 3-Scheme 1. This material was treated with hydrazine hydrate in a protic solvent (such as methanol or ethanol) to provide 4-Scheme 1. Treatment of 4-Scheme 1 with a carboxylic acid (such as N-benzyloxycarbonyl-L-valine, N-benzyloxycarbonyl-L-phenylalanine, N-benzyloxycarbonyl-L-isoleucine, N-benzyloxycarbonyl-L-methionine, N-benzyloxycarbonyl-L-norvaline, (S)-N-benzyloxycarbonyl-2-aminobutyric acid, N-*tert*-butoxycarbonyl-L-leucine, (R)-4-methyl-2-(3-phenylphenyl)pentanoic acid, N-

benzyloxycarbonyl-L-cyclohexylglycine or N-benzyloxycarbonyl-L-cyclohexylalanine and a peptide coupling reagent (such as EDC•HCl/1-HOBT) in an aprotic solvent (such as DMF) provided 5-Scheme-1. When R was *tert*-butyl, 5-Scheme-1 was converted to 6-Scheme 1 by treatment with trifluoroacetic acid in dichloromethane. Alternatively, when R was benzyl, 5-Scheme-1 was converted to 6-Scheme 1 by treatment with hydrobromic acid in acetic acid. Treatment of 6-Scheme 1 with a carboxylic acid (such as N_{α}-benzyloxycarbonyl-N_{ε}-*tert*-butoxycarbonyl-L-lysine, N_{α}-benzyloxycarbonyl-$N_{\phi 2}$-bis(*tert*-butoxycarbonyl)-L-arginine, 3,4-dimethoxybenzoic acid, 4-trifluoromethylbenzoic acid, 3,4-dichlorobenzoic acid, benzofuran-2-carboxylic acid, 5,6-dimethoxybenzofuran-2-carboxylic acid, 5-methyl-2-phenyloxazole-4-carboxylic acid, benzothiophene-2-carboxylic acid, 4-methyl-2-(4-trifluoromethylphenyl)thiazole-5-carboxylic acid, isoquinoline-3-carboxylic acid, 5-chlorobenzofuran-2-carboxylic acid 3,5-difluorobenzoic acid benzothiazole-6-carboxylic acid, picolinic acid, 2,6-dimethoxynicotinic acid, 4-methanesulfonylbenzoic acid, 3,4,5-trimethoxybenzoic acid, quinoline-8-carboxylic acid, 3,4-(1,3-propylenedioxy)benzoic acid, thieno[2,3-b]thiophene-2-carboxylic acid, 4-methoxybenzoinc acid, 3-[2-(4-morpholino)ethoxy]-4-methoxybenzoic acid, 2-(4-morpholino)pyrimidine-4-carboxylic acid, 2,3-dihydrobenzofuran-5-carboxylic acid, 5-carboxybenzofuran-2-carboxylic acid and a peptide coupling reagent (such as EDC•HCl/1-HOBT) in an aprotic solvent (such as DMF) provided 7-Scheme-1. Alternatively, 7-Scheme 1 was prepared by treatment of 6-Scheme 1 with an acyl chloride (such as 3-nitro-1,2,7-benzoxadiazol-6-yl-L-prolinyl chloride) and N-methylmorpholine in dichloromethane. When $R^{12}CO_2H$ was N_{α}-benzyloxycarbonyl-N_{ε}-*tert*-butoxycarbonyl-L-lysine or N_{α}-benzyloxycarbonyl-$N_{\phi 2}$-bis(*tert*-butoxycarbonyl)-L-arginine, the *tert*-butoxycarbonyl groups were removed by treatment with trifluoroacetic acid in dichloromethane.

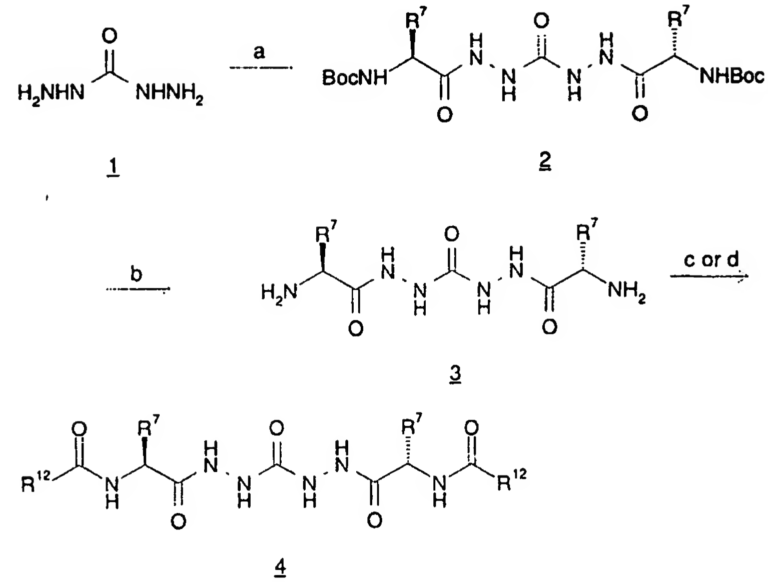
Scheme 2



a) BnOCOCl, CH_2Cl_2; b) $(t\text{-BuOCO})_2O$, dioxane; c) R^1CO_2H, EDC·HCl, 1-HOBT, DMF; d) HBr, HOAc; e) TFA, CH_2Cl_2; f) $COCl_2$, $R^6CONHNH_2$, NMM, CH_2Cl_2.

Compounds of the Formula I, wherin $R^1 \neq R^6$ and R^3 or $R^4 \neq$ H, are prepared by methods analogous to those described in Scheme 2. Treatment of 1-Scheme 2 with benzyl chloroformate in dichloromethane provided 2-Scheme 2. Alternatively, treatment of 1-Scheme 2 with di-*tert*-butyl dicarbonate in dioxane provided 2-Scheme 2. Treatment of 2-Scheme 2 with a carboxylic acid (such as N-benzyloxycarbonyl-L-leucine or 3-(2-pyridinyl)phenylacetic acid) and a peptide coupling reagent (such as EDC•HCl/1-HOBT) in an aprotic solvent (such as DMF) gave 3-Scheme 2. When R was benzyl, 3-Scheme 2 was converted to 4-Scheme 2 by treatment with hydrobromic acid in acetic acid. Alternatively, when R was *tert*-butyl, 3-Scheme 2 was converted to 4-Scheme 2 by treatment with trifluoroacetic acid in dichloromethane. Treatment of 4-Scheme 2 with phosgene and N-methylmorpholine in dichloromethane, followed by treatment with an acyl hydrazine (such as N-benzyloxycarbonyl-L-leucinylhydrazide or 3-(2-pyridinyl)phenylacetylhydrazide) and N-methylmorpholine in dichloromethane provided 5-Scheme 2.

Scheme 3



a) $BocNHCH(R^7)CO_2H$, EDC·HCl, 1-HOBT, DMF; b) TFA, CH_2Cl_2; c) $R^{12}CO_2H$, EDC·HCl, 1-HOBT, DMF; d) $R^{12}COCl$, NMM, CH_2Cl_2.

Compounds of the formula I wherin $R^1 = R^6 = R^{12}CONHCH(R^7)CO_2H$ are prepared by methods analogous to those described in Scheme 3. Treatment of 1-Scheme 3 with a *tert*-butoxycarbonyl-proteacted amino acid (such as *tert*-butoxycarbonyl-L-leucine) and a peptide coupling reagent (such as EDC•HCl/1-HOBT) in an aprotic solvent (such as DMF) provided 2-Scheme 3, which was treated with triflouroacetic acid in dichloromethane to give 3-Scheme 3. Treatment of 3-Scheme 3 with a carboxylic acid (such as 3-iodo-4-methoxybenzoic acid) and a peptide coupling reagent (such as EDC•HCl/1-HOBT) in an aprotic solvent (such as DMF) provided 4-Scheme 3. Alternatively, 4-Scheme 3 was prepared by treatment of 3-Scheme 3 with an acyl chloride (such as 3-nitro-1,2,7-benzoxadiazol-6-yl-L-prolinyl chloride) and N-methylmorpholine in dichloromethane.

Referring to the methods of preparing the compounds of Formula I set forth in Schemes 1-3 above, the skilled artisan will appreciate that the present invention includes all novel intermediates required to make the compounds of Formula I. More specifically, the present invention includes the following compounds:

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_α-benzyloxycarbonyl-N_ε-*tert*-butoxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_α-benzyloxycarbonyl-$N_{\phi 2}$-bis(*tert*-butoxycarbonyl)-L-lysinyl-L-leucinyl)]carbohydrazide;

2,2'-[N,N'-bis-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide;

2,2'-[N,N'-bis-(L-leucinyl)]carbohydrazide;

2-hydroxy-4,5-dimethoxybenzaldehyde;

4,5-dimethoxy-2-ethoxycarbonylmethoxybenzaldehyde;

ethyl 5,6-dimethoxybenzofuran-2-carboxylate;

5,6-dimethoxybenzofuran-2-carboxylic acid;

1-(N-benzyloxycarbonyl)-1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine;

1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine;

1-(N-tert-butoxycarbonyl)-1(-N-methyl)-2-(N-benzyloxycarbonyl-L-leucinyl)hydrazine;

2-(N-benzyloxycarbonyl-L-leucinyl)-1-(N-methyl)hydrazine;

3-(2-pyridyl)phenylacetylhydrazine;

ethyl 2-(4-morpholino)pyrimidine-4-carboxylate;

2-(4-morpholino)pyrimidine-4-carboxylic acid;

4-benzyloxycarbonylmethoxy-3-formylbenzyaldehyde;

benzyl 5-formylbenzofuran-2-carboxylate;

benzyl 5-carboxybenzofuran-2-carboxylate;

benzyl 5-*tert*-butoxycarbonylbenzofuran-2-carboxylate;

5-*tert*-butoxycarbonylbenzofuran-2-carboxylic acid;

2-[N-[N-(5-*tert*-butoxycarbonylbenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide; and

2-(N-L-leucinyl)-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide.

The starting materials used herein are commercially available amino acids or are prepared by routine methods well known to those of ordinary skill in the art and can be

found in standard reference books, such as the COMPENDIUM OF ORGANIC SYNTHETIC METHODS, Vol. I-VI (published by Wiley-Interscience).

Coupling methods to form amide bonds herein are generally well known to the art. The methods of peptide synthesis generally set forth by Bodansky *et al.*, THE PRACTICE OF PEPTIDE SYNTHESIS, Springer-Verlag, Berlin, 1984; E. Gross and J. Meienhofer, THE PEPTIDES, Vol. 1, 1-284 (1979); and J.M. Stewart and J.D. Young, SOLID PHASE PEPTIDE SYNTHESIS, 2d Ed., Pierce Chemical Co., Rockford, Ill., 1984. are generally illustrative of the technique and are incorporated herein by reference.

Synthetic methods to prepare the compounds of this invention frequently employ protective groups to mask a reactive functionality or minimize unwanted side reactions. Such protective groups are described generally in Green, T.W, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York (1981). The term "amino protecting groups" generally refers to the Boc, acetyl, benzoyl, Fmoc and Cbz groups and derivatives thereof as known to the art. Methods for protection and deprotection, and replacement of an amino protecting group with another moiety are well known.

Acid addition salts of the compounds of Formula I are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, hydrofluoric, sulfuric, phosphoric, acetic, trifluoroacetic, maleic, succinic or methanesulfonic. Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or alkoxide, containing the appropriate cation; or with an appropriate organic amine. Cations such as Li^+, Na^+, K^+, Ca^{++}, Mg^{++} and NH_4^+ are specific examples of cations present in pharmaceutically acceptable salts. Halides, sulfate, phosphate, alkanoates (such as acetate and trifluoroacetate), benzoates, and sulfonates (such as mesylate) are examples of anions present in pharmaceutically acceptable salts.

This invention also provides a pharmaceutical composition which comprises a compound according to Formula I and a pharmaceutically acceptable carrier, diluent or excipient. Accordingly, the compounds of Formula I may be used in the manufacture of a medicament. Pharmaceutical compositions of the compounds of Formula I prepared as hereinbefore described may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation may be a

buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water or buffered sodium or ammonium acetate solution. Such formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler or nebulizer for insufflation. It may be desirable to add excipients such as polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

Alternately, these compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Liquid carriers include syrup, peanut oil, olive oil, saline and water. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 g per dosage unit. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

For rectal administration, the compounds of this invention may also be combined with excipients such as cocoa butter, glycerin, gelatin or polyethylene glycols and molded into a suppository.

Utility of the Present Invention

The compounds of Formula I are useful as protease inhibitors, particularly as inhibitors of cysteine and serine proteases, more particularly as inhibitors of cysteine proteases, even more particularly as inhibitors of cysteine proteases of the papain superfamily, yet more particularly as inhibitors of cysteine proteases of the cathepsin family, most particularly as inhibitors of cathepsin K. The present invention also provides useful compositions and formulations of said compounds, including pharmaceutical compositions and formulations of said compounds.

The present compounds are useful for treating diseases in which cysteine proteases are implicated, including infections by pneumocystis carinii, trypsanoma cruzi, trypsanoma brucei, and Crithidia fusiculata; as well as in schistosomiasis, malaria, tumor metastasis, metachromatic leukodystrophy, muscular dystrophy, amytrophy; and especially diseases in which cathepsin K is implicated, most particularly diseases of excessive bone or cartilage loss, including osteoporosis, gingival disease including gingivitis and periodontitis, arthritis, more specifically, osteoarthritis and rheumatoid arthritis, Paget's disease; hypercalcemia of malignancy, and metabolic bone disease.

Metastatic neoplastic cells also typically express high levels of proteolytic enzymes that degrade the surrounding matrix, and certain tumors and metastatic neoplasias may be effectively treated with the compounds of this invention.

The present invention also provides methods of treatment of diseases caused by pathological levels of proteases, particularly cysteine and serine proteases, more particularly cysteine proteases, even more particularly as inhibitors of cysteine proteases of the papain superfamily, yet more particularly cysteine proteases of the cathepsin family, which methods comprise administering to an animal, particularly a mammal, most particularly a human in need thereof a compound of the present invention. The present invention especially provides methods of treatment of diseases caused by pathological levels of cathepsin K, which methods comprise administering to an animal, particularly a mammal, most particularly a human in need thereof an inhibitor of cathepsin K, including a compound of the present invention. The present invention particularly provides methods for treating diseases in which cysteine proteases are implicated, including infections by pneumocystis carinii, trypsanoma cruzi, trypsanoma brucei, and Crithidia fusiculata; as well as in schistosomiasis, malaria, tumor metastasis, metachromatic leukodystrophy, muscular dystrophy, amytrophy, and especially diseases in which cathepsin K is implicated, most particularly diseases of excessive bone or cartilage loss, including osteoporosis, gingival disease including gingivitis and periodontitis, arthritis, more specifically, osteoarthritis and rheumatoid arthritis, Paget's disease, hypercalcemia of malignancy, and metabolic bone disease.

This invention further provides a method for treating osteoporosis or inhibiting bone loss which comprises internal administration to a patient of an effective amount of a compound of Formula I, alone or in combination with other inhibitors of bone resorption, such as bisphosphonates (i.e., allendronate), hormone replacement therapy, anti-estrogens,

or calcitonin. In addition, treatment with a compound of this invention and an anabolic agent, such as bone morphogenic protein, iproflavone, may be used to prevent bone loss or to increase bone mass.

For acute therapy, parenteral administration of a compound of Formula I is preferred. An intravenous infusion of the compound in 5% dextrose in water or normal saline, or a similar formulation with suitable excipients, is most effective, although an intramuscular bolus injection is also useful. Typically, the parenteral dose will be about 0.01 to about 100 mg/kg; preferably between 0.1 and 20 mg/kg, in a manner to maintain the concentration of drug in the plasma at a concentration effective to inhibit cathepsin K. The compounds are administered one to four times daily at a level to achieve a total daily dose of about 0.4 to about 400 mg/kg/day. The precise amount of an inventive compound which is therapeutically effective, and the route by which such compound is best administered, is readily determined by one of ordinary skill in the art by comparing the blood level of the agent to the concentration required to have a therapeutic effect.

The compounds of this invention may also be administered orally to the patient, in a manner such that the concentration of drug is sufficient to inhibit bone resorption or to achieve any other therapeutic indication as disclosed herein. Typically, a pharmaceutical composition containing the compound is administered at an oral dose of between about 0.1 to about 50 mg/kg in a manner consistent with the condition of the patient. Preferably the oral dose would be about 0.5 to about 20 mg/kg.

No unacceptable toxicological effects are expected when compounds of the present invention are administered in accordance with the present invention.

Biological Assays

The compounds of this invention may be tested in one of several biological assays to determine the concentration of compound which is required to have a given pharmacological effect.

Determination of cathepsin K proteolytic catalytic activity

All assays for cathepsin K were carried out with human recombinant enzyme. Standard assay conditions for the determination of kinetic constants used a fluorogenic peptide substrate, typically Cbz-Phe-Arg-AMC, and were determined in 100 mM Na acetate at pH 5.5 containing 20 mM cysteine and 5 mM EDTA. Stock substrate solutions

were prepared at concentrations of 10 or 20 mM in DMSO with 20 uM final substrate concentration in the assays. All assays contained 10% DMSO. Independent experiments found that this level of DMSO had no effect on enzyme activity or kinetic constants. All assays were conducted at ambient temperature. Product fluorescence (excitation at 360 nM; emission at 460 nM) was monitored with a Perceptive Biosystems Cytofluor II fluorescent plate reader. Product progress curves were generated over 20 to 30 minutes following formation of AMC product.

Inhibition studies

Potential inhibitors were evaluated using the progress curve method. Assays were carried out in the presence of variable concentrations of test compound. Reactions were initiated by addition of enzyme to buffered solutions of inhibitor and substrate. Data analysis was conducted according to one of two procedures depending on the appearance of the progress curves in the presence of inhibitors. For those compounds whose progress curves were linear, apparent inhibition constants ($K_{i,app}$) were calculated according to equation 1 (Brandt *et al.*, *Biochemitsry*, **1989**, *28*, 140):

$$v = V_m A / [K_a(1 + I/K_{i,\ app}) + A] \tag{1}$$

where v is the velocity of the reaction with maximal velocity V_m , A is the concentration of substrate with Michaelis constant of K_a, and I is the concentration of inhibitor.

For those compounds whose progress curves showed downward curvature characteristic of time-dependent inhibition, the data from individual sets was analyzed to give k_{obs} according to equation 2:

$$[\text{AMC}] = v_{ss}\, t + (v_0 - v_{ss})\, [1 - exp\,(-k_{obs}t)] / k_{obs} \tag{2}$$

where [AMC] is the concentration of product formed over time t, v_0 is the initial reaction velocity and v_{ss} is the final steady state rate. Values for k_{obs} were then analyzed as a linear function of inhibitor concentration to generate an apparent second order rate

constant (k_{obs} / inhibitor concentration or k_{obs} / [I]) describing the time-dependent inhibition. A complete discussion of this kinetic treatment has been fully described (Morrison *et al.*, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **1988**, *61*, 201).

Human Osteoclast Resorption Assay

Aliquots of osteoclastoma-derived cell suspensions were removed from liquid nitrogen storage, warmed rapidly at 37°C and washed x1 in RPMI-1640 medium by centrifugation (1000 rpm, 5 min at 4°C). The medium was aspirated and replaced with murine anti-HLA-DR antibody, diluted 1:3 in RPMI-1640 medium, and incubated for 30 min on ice The cell suspension was mixed frequently.

The cells were washed x2 with cold RPMI-1640 by centrifugation (1000 rpm, 5 min at 4°C) and then transferred to a sterile 15 mL centrifuge tube. The number of mononuclear cells were enumerated in an improved Neubauer counting chamber.

Sufficient magnetic beads (5 / mononuclear cell), coated with goat anti-mouse IgG, were removed from their stock bottle and placed into 5 mL of fresh medium (this washes away the toxic azide preservative). The medium was removed by immobilizing the beads on a magnet and is replaced with fresh medium.

The beads were mixed with the cells and the suspension was incubated for 30 min on ice. The suspension was mixed frequently. The bead-coated cells were immobilized on a magnet and the remaining cells (osteoclast-rich fraction) were decanted into a sterile 50 mL centrifuge tube. Fresh medium was added to the bead-coated cells to dislodge any trapped osteoclasts. This wash process was repeated x10. The bead-coated cells were discarded.

The osteoclasts were enumerated in a counting chamber, using a large-bore disposable plastic pasteur pipette to charge the chamber with the sample. The cells were pelleted by centrifugation and the density of osteoclasts adjusted to 1.5×10^4/mL in EMEM medium, supplemented with 10% fetal calf serum and 1.7g/litre of sodium bicarbonate. 3 mL aliquots of the cell suspension (per treatment) were decanted into 15 mL centrifuge tubes. These cells were pelleted by centrifugation. To each tube 3 mL of the appropriate treatment was added (diluted to 50 uM in the EMEM medium). Also included were appropriate vehicle controls, a positive control (87MEM1 diluted to 100 ug/mL) and an isotype control (IgG2a diluted to 100 ug/mL). The tubes were incubate at 37°C for 30 min.

0.5 mL aliquots of the cells were seeded onto sterile dentine slices in a 48-well plate and incubated at 37°C for 2 h. Each treatment was screened in quadruplicate. The slices were washed in six changes of warm PBS (10 mL / well in a 6-well plate) and then placed into fresh treatment or control and incubated at 37°C for 48 h. The slices were then washed in phosphate buffered saline and fixed in 2% glutaraldehyde (in 0.2M sodium cacodylate) for 5 min., following which they were washed in water and incubated in buffer for 5 min at 37°C. The slices were then washed in cold water and incubated in cold acetate buffer / fast red garnet for 5 min at 4°C. Excess buffer was aspirated, and the slices were air dried following a wash in water.

The TRAP positive osteoclasts were enumerated by bright-field microscopy and were then removed from the surface of the dentine by sonication. Pit volumes were determined using the Nikon/Lasertec ILM21W confocal microscope.

General

Nuclear magnetic resonance spectra were recorded at either 250 or 400 MHz using, respectively, a Bruker AM 250 or Bruker AC 400 spectrometer. $CDCl_3$ is deuteriochloroform, DMSO-d_6 is hexadeuteriodimethylsulfoxide, and CD_3OD is tetradeuteriomethanol. Chemical shifts are reported in parts per million (d) downfield from the internal standard tetramethylsilane. Abbreviations for NMR data are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, app = apparent, br = broad. J indicates the NMR coupling constant measured in Hertz. Continuous wave infrared (IR) spectra were recorded on a Perkin-Elmer 683 infrared spectrometer, and Fourier transform infrared (FTIR) spectra were recorded on a Nicolet Impact 400 D infrared spectrometer. IR and FTIR spectra were recorded in transmission mode, and band positions are reported in inverse wavenumbers (cm^{-1}). Mass spectra were taken on either VG 70 FE, PE Syx API III, or VG ZAB HF instruments, using fast atom bombardment (FAB) or electrospray (ES) ionization techniques. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. All temperatures are reported in degrees Celsius.

Analtech Silica Gel GF and E. Merck Silica Gel 60 F-254 thin layer plates were used for thin layer chromatography. Both flash and gravity chromatography were carried out on E. Merck Kieselgel 60 (230-400 mesh) silica gel.

Where indicated, certain of the materials were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin, Chemical Dynamics Corp., South Plainfield, New Jersey, and Advanced Chemtech, Louisville, Kentucky.

Examples

In the following synthetic examples, temperature is in degrees Centigrade (°C). Unless otherwise indicated, all of the starting materials were obtained from commercial sources. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. These Examples are given to illustrate the invention, not to limit its scope. Reference is made to the claims for what is reserved to the inventors hereunder.

Example 1

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-valinyl)]carbohydrazide

a) methyl 3-benzyloxybenzoate

To a mixture of methyl 3-hydroxybenzoate (10.0 g, 65.7 mmol) and potassium carbonate (13.6 g, 98.6 mmol) in acetone (125 mL) was added benzyl bromide (11.2 g, 65.7 mmol). After stirring at reflux for 16h, the mixture was partitioned between ethyl acetate and water. The organic phase was washed with brine, dried ($MgSO_4$) filtered and concentrated to yield the title compound as a white solid (15.57 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) d 7.67 (m, 2H), 7.48 - 7.35 (m, 6H), 7.19 (m, 1H), 5.02 (s, 2H), 3.93 (s, 3H).

b) 3-benzyloxybenzoyl hydrazide

To a stirring solution of the compound of Example 1(a) (10.0 g, 41.3 mmol) in 100 mL of methanol was added hydrazide hydrate (21.0 g, 413 mmol). The solution was stirred at room temperature for 16h, then concentrated to yield the title compound as an off-white solid (9.56 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) d 7.45 - 7.27 (m, 9H), 7.24 (m, 1H), 5.12 (s, 2H), 4.10 (s b, 2H).

c) 5-(3-benzyloxyphenyl)-1,3,4-oxadiazol-2-one

To a stirring solution of the compound of Example 1(b) (7.0 g, 28.9 mmol) in toluene (125 mL) was added phosgene (45 mL, 1.93M in toluene). The solution was heated at reflux for 4h then concentrated to yield the title compound as a pale yellow foam (7.45 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) d 7.41 - 7.28 (m, 9H), 7.06 (m, 1H), 5.06 (s, 2H).

d) 2-[N-(3-benzyloxybenzoyl)]carbohydrazide

Following the procedure of Example 1(b), except substituting 5-(3-benzyloxyphenyl)-1,3,4-oxadiazol-2-one for methyl 3-benzyloxybenzoate, the title compound was prepared as a white foam (7.73 g, 93%). MS (ESI): 301.1 $(M+H)^+$.

e) 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-valinyl)]carbohydrazide

To a stirring solution of the compound of Example 1(d) (0.125g, 0.417mmol), N-benzyloxycarbonyl-L-valine (0.096 g, 0.458 mmol), and 1-hydroxybenzotriazole (0.011 g, 0.0834 mmol) in DMF (5 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.088 g, 0.458 mmol). After stirring at room temperature for 16h, the solution was poured into water (50 mL). The precipitate was collected by filtration and washed several times with water then hexanes. The solid was allowed to air dry for 2h to yield the title compound as a white solid (0.183 g, 89%). MS (ESI): 492.3 $(M+H)^+$.

Example 2

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-phenylalanyl)]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting benzyloxycarbonyl-L-phenylalanine for benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.186 g, 77%). MS (ESI): 604.3 $(M+Na)^+$.

Example 3

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-isoleucinyl)]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting benzyloxycarbonyl-L-isoleucine for benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.204 g, 89%). MS (ESI): 570.3 $(M+Na)^+$.

Example 4

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-methionyl)]carbohydrazide

Following the procedure of 1(a)-1(e), except substituting benzyloxycarbonyl-L-methionine for benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.200 g, 85%). MS (ESI): 566.4 $(M+H)^+$.

Example 5

Preparation of 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide

a) N-benzyloxycarbonyl-L-leucine methyl ester

To a stirring solution of L-leucine methyl ester hydrochloride (2.0 g, 11.0 mmol) in 1,4-dioxane (20 mL) was added Na_2CO_3 (12.1 ml, 2M in water) followed by benzylchloroformate (1.96 g, 11.5 mmol). The mixture was stirred at room temperature for 4h then partitioned between ethyl acetate and water. The organic layer was washed with brine, dried ($MgSO_4$) filtered and concentrated to yield the title compound as a colorless oil (3.1 g, 100%). ^1H NMR (400 MHz, $CDCl_3$) d 7.34 (m, 5H), 5.27 (d, 1H), 5.12 (s, 2H), 4.41 (s, 2H), 3.75 (s, 3H), 1.65 (m, 3H), 0.96 (m, 6H).

b) N-benzyloxycarbonyl-L-leucinylhydrazide

Following the procedure of Example 1(b), except substituting N-benzyloxycarbonyl-L-leucine methyl ester for methyl 3-benzyloxybenzoate, the title compound was prepared as an off-white solid (3.1 g, 100%). MS(ESI): 280.2 $(M+H)^+$.

c) 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide

Following the procedure of 1(c)-1(e), except substituting N-benzyloxycarbonyl-L-leucine methyl ester for methyl 3-benzyloxybenzoate in step (b), and N-benzyloxycarbonyl-L-norvaline for N-benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.085 g, 40%). MS(ESI): 571.3 $(M+H)^+$.

Example 6

Preparation of (2S)-2-[N-(N-benzyloxycarbonyl-2-aminobutyryl)]-2'-[N'-(N-benzyloxycarbonyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 5(a)-5(c), except substituting (S)-N-benzyloxycarbonyl-2-aminobutyric acid for N-benzyloxycarbonyl-L-norvaline in step (c), the title compound was prepared as a white solid (0.065g, 32%). MS (ESI): 579.3 $(M+Na)^+$.

Example 7

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide

a) 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 1(e), except substituting N-*tert*-butoxycarbonyl-L-leucine for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.800 g, 94%). MS (ESI): 536.3 $(M+Na)^+$.

b) 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(L-leucinyl)]carbohydrazide

To a stirring solution of the compound of Example 7(a) (0.800 g, 1.55 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (4 mL). After stirring at room temperature for 2hr, the solution was concentrated. The residue was dissolved in ethyl acetate and washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated to yield the title compound as a white solid (0.640 g, 100%). MS(ESI): 414.2 $(M+H)^+$.

c) 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_α-benzyloxycarbonyl-N_ε-*tert*-butoxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 1(e), except substituting N_α-benzyloxycarbonyl-N_ε-*tert*-butoxycarbonyl-L-lysine for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.158 g, 67%). MS (ESI): 799.4 $(M+Na)^+$.

d) 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 7(b), except substituting 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_α-benzyloxycarbonyl-N_ε-*tert*-butoxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide, the title compound was prepared as a white solid (0.126 g, 93%). MS (ESI): 676.5 $(M+H)^+$.

Example 8

Preparation of (2R)-2-[N-(3-benzyloxybenzoyl)]-2'-[N'-[4-methyl-2-(3-phenylphenyl)pentanoyl]carbohydrazide

a) methyl 3-bromophenylacetate

3-Bromophenyl acetic acid (2.15 g, 10 mmol) was dissolved in ether, then was treated with a solution of diazomethane until the yellow color persisted. The reaction was then quenched with AcOH, concentrated in vacuo and was used in the next reaction without further purification.

b) methyl 3-phenylphenylacetate

The compound of Example 8(a) (2.29 g, 10 mmol) was dissolved in toluene (30 mL). Then, phenylboronic acid (1.46 g, 12 mmol) was added followed by aqueous sodium carbonate (2M, 4.24 mL, 40 mmol), then tetrakis(triphenylphosphine) palladium(0) (0.35 g, 0.3 mmol) and the mixture was heated at reflux overnight. The reaction was cooled to room temperature, diluted with saturated ammonium chloride, then extracted with EtOAc (2 x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated, and chromatographed (silica gel, 5% EtOAc: hexanes) to provide the title compound as a white solid (1.93 g, 84%). MS (ESI): 263 $(M+H)^+$.

c) 3-phenylphenylacetic acid

The compound of Example 8(b) (1.90 g, 8.4 mmol) was dissolved in MeOH (40 mL) and water (6 mL), then $LiOH \cdot H_2O$ (0.7 g, 16.8 mmol) was added, and the reaction was stirred at room temperature for 2h. The reaction was diluted with water, acidified with 6N HCl (1 mL), then extracted with EtOAc (2 x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give the title compound as a white solid (1.66 g, 93%). ^1H NMR (400 MHz, $CDCl_3$) d 7.6-7.25 (m, 9H), 3.7 (s, 2H).

d) 4-methyl-2-(3-phenylphenyl)pent-4-enoic acid

n-BuLi (5.2 mmol, 3.26 mL, 1.6 M in hexanes) was added dropwise to a solution of diisopropyl amine (0.54 g, 5.3 mmol, 0.74 mL) in THF (6 mL) at 0 °C. The reaction was allowed to stir for 15 minutes, then was cooled to -78 °C. A solution of the compound of Example 8(c) (0.5g, 2.35 mmol) in THF (2 mL) and was added dropwise. The reaction was warmed to 0 °C, allowed to stir for 40 min, then cooled to -78 °C. 3-bromo-2-methylpropene (0.475 g, 3.52 mmol) was added and the reaction was allowed to stir for 1h. Water (2 mL) was added and the volatiles was removed in vacuo. The mixture was diluted with water, acidified with 6N HCl (1 mL), then extracted with EtOAc (2 x 10 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated, chromatographed (silica gel, 5% MeOH: methylene chloride) to give the title compound as a white solid (0.582 g, 93%). ^1H NMR (400 MHz, $CDCl_3$) d 7.6-7.3 (m, 9H), 4.75 (d, 2H), 3.87 (t, 1H), 2.87 (dd, 1H), 2.50 (dd, 1H), 1.70 (s, 3H).

e) 4-methyl-2-(3-phenylphenyl)pentanoic acid

The compound of Example 8(d) (0.5 g, 1.87 mmol) was dissolved in EtOAc (25 mL). Then, 10% Pd/C (60 mg) was added and the reaction was allowed to stir for 2.5 h under a balloon of hydrogen gas. The mixture was filtered, concentrated in vacuo, then was redissolved in 1:5 EtOAc:EtOH (15 mL). Then, 10% Pd/C (80 mg) was added and the reaction was stirred under a balloon of hydrogen gas overnight. The mixture was filtered, concentrated in vacuo, and chromatographed (silica gel, 5% MeOH: methylene chloride) to give the desired product as a white solid (0.5 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) d 7.6-7.3 (m, 9H), 3.7 (t, 1H), 2.07-1.95 (m, 1H), 1.8-1.7 (m, 1H), 1.6-1.45 (m, 1H).

f) (R)-4-methyl-2-(3-phenylphenyl)pentanoic acid

The compound of Example 8(e) (16.6 g, 62 mmol) was dissolved in EtOH (100 mL) and EtOAc (200 mL). (S)-*p*-bromo-α-methylbenzylamine (12.31 g, 62 mmol) was added and the solution was heated at 65 °C until the solid was completely dissolved. The solution was cooled in a refrigerator and white crystals formed overnight. The crystals were collected then were dried in vacuo. Four recrystallizations from a 1:2 EtOAc/EtOH yielded crystalline white solid (3.05 g, 21% recovery). Chiral HPLC indicated an enantiomeric ratio of 99.3% (R) and 0.7% (S). The solid was then dissolved in EtOAc, extracted with 1 N aqueous HCl, and the combined organic layers were dried ($MgSO_4$), filtered, concentrated in vacuo and was used in the next reaction without further purification.

g) (2R)-2-[N-(3-benzyloxybenzoyl)]-2'-[N'-[4-methyl-2-(3-phenylphenyl)pentanoyl]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting (R)-2-(3-phenylphenyl)-4-methylpentanoic acid for N-benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.106 g, 36%). MS (ESI): 573.3 $(M+Na)^+$.

Example 9

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-arginyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 7(a)-7(d), except substituting N_α-benzyloxycarbonyl-$N_{\phi 2}$-bis(*tert*-butoxycarbonyl)-L-arginine for N_α-benzyloxycarbonyl-N_ε-*tert*-butoxycarbonyl-L-lysine in step (c), the title compound was prepared as a white solid (0.364 g, 100%). MS (ESI): 704.4 $(M+H)^+$.

Example 10

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting N-benzyloxycarbonyl-L-norvaline for N-benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.195 g, 88%). MS (ESI): 556.2 $(M+Na)^+$.

Example 11

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylglycinyl)]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting N-benzyloxycarbonyl-L-cyclohexylglycine for N-benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.217 g, 91%). MS (ESI): 596.2 $(M+Na)^+$.

Example 12

Preparation of 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylalanyl)]carbohydrazide

Following the procedure of Example 1(a)-1(e), except substituting N-benzyloxycarbonyl-L-cyclohexylalanine for N-benzyloxycarbonyl-L-valine in step (e), the title compound was prepared as a white solid (0.246 g, 80%). MS (ESI): 588.5 $(M+H)^+$.

Example 13

Preparation of 2,2'-[N,N'-bis[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide

a) 2,2'-[N,N'-bis-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide

To a stirring solution of carbohydrazide (0.200 g, 2.22 mmol), N-*tert*-butoxycarbonyl-L-leucine (1.2 g, 4.88 mmol), and 1-hydroxybenzotriazole (0.060g, 0.444mmol) in DMF (20mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.935 g, 4.88 mmol). After stirring at room temperature for 16h, the solution was poured into water (200 mL). The precipitate was filtered off and washed with water and hexanes. The solid was allowed to air dry for 2h to yield the title compound as a white solid (0.993 g, 86%). MS (ESI): 539.4 $(M+Na)^+$.

b) 2,2'-[N,N'-bis-(L-leucinyl)]carbohydrazide bis(trifluoroacetate) salt

Following the procedure of Example 7(b), except substituting 2,2'-[N,N'-bis-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide, the title compound was prepared and as a white solid and was used without further characterization (1.0 g, 100%).

c) 2,2'-[N,N'-bis[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide

To a stirring solution of the compound of Example 13(b) (0.021 g, 0.0383 mmol) and N-methylmorpholine (0.019 g, 0.192 mmol) in dichloromethane (2 mL) and DMF (0.50 mL) was added N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl chloride (0.025 g, 0.0843 mmol). After stirring at room temperature for 16h, the solution was diluted with ethyl acetate and washed successively with saturated aqueous sodium bicarbonate, water (2x) and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel; methanol/dichloromethane) to yield the title compound as an orange solid (0.007 g, 22%). MS (ESI): 837.4 $(M+H)^+$.

Example 14

Preparation of 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) methyl 3-(trifluoromethylsulfonyloxy)phenylacetate

To an oven-dried flask under argon atmosphere containing sodium hydride (2.54 g, 60% dispersion in mineral oil, 63.5 mmol) was added anhydrous pentane (20 mL). The slurry was allowed to stir for 5 min, allowed to settle, most of the pentane was removed, and anhydrous THF (40 mL) was added. To this suspension was added a solution of methyl 3-hydroxyphenylacetate (9.99 g, 60.1 mmol) in anhydrous THF (20 mL) and the reaction was allowed to stir at room temperature for 20 min. To this mixture was then added a solution of N-phenyltrifluoromethanesulfonimide (22.53 g, 63.1 mmol)) in anhydrous THF (40 mL) and the reaction was allowed to stir at room temperature until TLC analysis indicated the complete consumption of starting material (1.5 h). The reaction was quenched by the addition of H_2O (10 mL), concentrated to one half original volume, then diluted with $CHCl_3$ (200 mL) and washed with H_2O. The aqueous layer was washed with fresh $CHCl_3$ (50 mL), the combined organic layers were washed with 10% Na_2CO_3, water, and saturated brine, then dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue (silica gel, 5:95 EtOAc: hexanes, then 10:90 EtOAc: hexanes) gave 17.47 g of the title compound. ^1H NMR (400 MHz, $CDCl_3$) 7.42 (m, 1H), 7.31-7.19 (m, 3H), 3.72 (s, 3H), 3.68 (s, 2H).

b) methyl 3-(2-pyridyl)phenylacetate

To a solution of the compound of Example 14(a) (6.86 g, 23.0 mmol) in anhydrous dioxane (100 mL) was added 2-pyridyltributylstannane (8.89 g, 24.1 mmol), LiCl (2.94 g, 69.3 mmol), 2,6-di-*tert*-butyl-4-methylphenol (a few crystals), and $Pd(PPh_3)_4$ (632.1 mg, 0.55 mmol). The reaction was protected from light with foil and heated at reflux overnight. The reaction was allowed to cool to room temperature and was concentrated. Column chromatography of the residue (silica gel, 1:3 EtOAc: hexanes, then 1:2 EtOAc: hexanes) gave 3.85 g of the title compound. MS (ESI): 228.1 $(M+H)^+$.

c) 3-(2-pyridyl)phenylacetic acid

To a solution of the compound of Example 14(b) (3.8 g, 16.7 mmol) in THF (50 mL) was added a solution of $LiOH{\bullet}H_2O$ (780.2 mg, 18.6 mmol) in water (10 mL). The reaction was allowed to at room temperature until TLC analysis indicated the complete consumption of starting material (2 h). The reaction mixture was concentrated to remove THF, then neutralized to pH 7 by the addition of 1N HCl, diluted with brine (50 mL), and washed with $CHCl_3$ (100 mL) The aqueous layer was readjusted back to pH 7 by the addition on 1N NaOH and washed with fresh $CHCl_3$ (100 mL). After repeating this procedure once more, the organic layers were combined, dried ($MgSO_4$), filtered and concentrated to give 3.79 g of the title compound. MS (ESI): 214.3 $(M+H)^+$.

d) 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 5(a)-5(c), except substituting 3-(2-pyridyl)phenylacetic acid for N-benzyloxycarbonyl-L-norvaline in step (c), the title compound was prepared as a white solid (1.03 g, 65%). MS (ESI): 533.3 $(M+H)^+$.

Example 15

Preparation of 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

To a stirring solution of 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide (1.5 g, 2.8 mmol) in acetic acid (15 mL) was added

30% HBr in acetic acid (45 mL). After stirring at room temperature for 1h, diethyl ether was added (500 mL) and the precipitate was filtered off. The precipitate was dried under high vacuum to yield the title compound as a pale pink solid (1.4 g, 89%). MS (ESI): 399.4 $(M+H)^+$.

Example 16

Preparation of 2-[N-[N-(3,4-dimethoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 3,4-dimethoxybenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as white solid (0.038 g; 30%). MS (ESI): 563.2 $(M+H)^+$.

Example 17

Preparation of 2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(4-trifluoromethylbenzoyl)-L-leucinyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 4-trifluoromethylbenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.057 g, 45%). MS (ESI): 571.2 $(M+H)^+$.

Example 18

Preparation of 2-[N-[N-(3,4-dichlorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 3,4-dichlorobenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.087 g, 68%). MS (ESI): 571.2 $(M+H)^+$.

Example 19

Preparation of 2-[N-(N-benzofuran-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and benzofuran-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.078 g, 65%). MS (ESI): 543.2 $(M+H)^+$.

Example 20

Preparation of 2-[N-[N-(5,6-dimethoxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) 2-hydroxy-4,5-dimethoxybenzaldehyde

To a stirring solution of 2-benzyloxy-4,5-dimethoxybenzaldehyde (1.0 g, 3.67 mmol) in ethyl acetate (25 mL) was added 10% palladium on carbon (0.50 g). The mixture was stirred under a hydrogen atmosphere for 4h, then filtered through Celite. The filtrate was concentrated to yield the title compound as a pale yellow solid (0.632 g, 95%). ^1H

NMR (400 MHz, $CDCl_3$) d 11.41 (s, 1H), 9.72 (s, 1H), 6.89 (s, 1H), 6.48 (s, 1H), 3.91 (s, 3H), 3.88 (s, 3H).

b) 4,5-dimethoxy-2-ethoxycarbonylmethoxybenzaldehyde

Following the procedure of Example 1(a), except substituting 2-hydroxy-4,5-dimethoxybenzaldehyde for methyl 3-hydroxybenzoate and ethyl bromoacetate for benzyl bromide, the title compound was prepared (0.758 g, 82%). ^1H NMR (400 MHz, $CDCl_3$) d 10.39 (s, 1H), 7.30 (s, 1H), 6.41 (s, 1H), 4.72 (s, 2H), 4.22 (q, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 1.26 (t, 3H).

c) ethyl 5,6-dimethoxybenzofuran-2-carboxylate

A mixture of the compound of Example 20(b) (0.758 g, 2.8 mmol) and potassium carbonate (0.975 g, 7.1 mmol) was stirred at 80°C in DMF (20 mL) for 5h. The mixture was cooled and partitioned between ethyl acetate and water. The organic layer was washed with water and brine then dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel, ethyl acetate/hexane) to yield the title compound as a white solid (0.405 g, 58%). ^1H NMR (400 MHz, $CDCl_3$) d 7.45 (s, 1H), 7.10 (s, 1H), 7.04 (s, 1H), 4.41 (q, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 1.41 (t, 3H).

d) 5,6-dimethoxybenzofuran-2-carboxylic acid

Following the procedure of Example 14(c), except substituting ethyl 5,6-dimethoxybenzofuran-2-carboxylate for methyl 3-(2-pyridyl)phenylacetate, the title compound was prepared as a white solid (0.263 g, 73%). ^1H NMR (400 MHz, $CDCl_3$) d 7.40 (s, 1H), 7.03 (s, 1H), 7.01 (s, 1H), 3.90 (s, 3H), 3.88 (s, 3H).

e) 2-[N-[N-(5,6-dimethoxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 5,6-dimethoxybenzofuran-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.110 g, 68%). MS (ESI): 603.2 $(M+H)^+$.

Example 21

Preparation of 2-[N-[N-(5-methyl-2-phenyloxazol-4-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 5-methyl-2-phenyloxazole-4-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.107 g, 68%). MS (ESI): 584.3 $(M+H)^+$.

Example 22

Preparation of 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1'-N'-methyl-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) 1-(N-benzyloxycarbonyl)-1-(N-methyl)hydrazine

To a stirring solution of methylhydrazine (10.6 g, 230 mmol) in dichloromethane (100 mL) at 0°C was added benzylchloroformate (3.9 g, 23 mmol) dropwise. After stirring at room temperature for 1h, the solution was concentrated and the residue purified by column chromatography (silica gel; ethyl acetate/hexane) to yield the title compound as a colorless oil (3.9 g, 94%). MS (ESI): 181.0 $(M+H)^+$.

b) 1-(N-benzyloxycarbonyl)-1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine

Following the procedure of Example 1(e), except substituting 1-(N-benzyloxycarbonyl)-1-(N-methyl)hydrazine for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 3-(2-pyridinyl)phenylacetic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.373 g, 96%). MS (ESI): 376.3 $(M+H)^+$.

c) 1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine bis(hydrobromide) salt

Following the procedure of Example 15, except substituting 1-(N-benzyloxycarbonyl)-1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine for 2-[N-(N-

benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide, the title compound was prepared as a light orange solid (0.454 g). MS (ESI): 242.1 $(M+H)^+$.

d) 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1'-N'-methyl-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

To a solution of phosgene (0.327 mL, 1.93 M in toluene) in dichloromethane (2 mL) at 0°C was added dropwise a solution of the compound of Example 22(c) (0.200 g, 0.497 mmol) and N-methylmorpholine (0.161 g, 1.6 mmol) in dichloromethane (2 mL). After stirring for 15 min, a solution of the compound of Example 5(b) (0.139 g, 0.497 mmol) and N-methylmorpholine (0.055 g, 0.547 mmol) in dichloromethane (2 mL) was added dropwise. After stirring at room temperature overnight, the solution was diluted with ethyl acetate and washed successively with saturated aqueous sodium bicarbonate, water (2x) and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel; methanol/dichloromethane) to yield the title compound as white solid (0.028 g, 10%). MS (ESI): 547.3 $(M+H)^+$.

Example 23

Preparation of 2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and benzothiophene-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.089 g, 60%). MS (ESI): 559.1 $(M+H)^+$.

Example 24

Preparation of 2-[N-[N-[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 4-methyl-2-(4-trifluoromethylphenyl)thiazole-5-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.074 g, 41%). MS (ESI): 668.1 $(M+H)^+$.

Example 25

Preparation of 2-[N-[N-(3-isoquinolinoyl)-L-leucinyl]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and isoquinoline-3-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.067 g, 45%). MS (ESI): 554.3 $(M+H)^+$.

Example 26

Preparation of 2-[N-[N-(5-chlorobenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 5-chlorobenzofuran-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.035 g, 23%). MS (ESI): 577.1 $(M+H)^+$.

Example 27

Preparation of 2-[N-[N-(3,5-difluorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 3,5-difluorobenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.064 g, 44%). MS (ESI): 539.3 $(M+H)^+$.

Example 28

Preparation of 2-[N-(N-benzothiazol-6-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and benzothiazole-6-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.078 g, 52%). MS (ESI): 560.1 $(M+H)^+$.

Example 29

Preparation of 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1-(N-methyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) 1-(N-*tert*-butoxycarbonyl)-1-(N-methyl)hydrazine

To a stirring solution of methylhydrazine (0.866 g, 18.8 mmol) in dioxane (1.8 mL) at 0°C was slowly added a solution of di-*tert*-butyldicarbonate (2.05 g, 9.4 mmol) in dioxane (2.8 mL). After stirring at room temperature for 2h, the mixture was filtered and the filtrate concentrated to yield the title compound as a colorless oil (0.258 g). MS (ESI): 146.8 $(M+H)^+$.

b) 1-(N-*tert*-butoxycarbonyl)-1(-N-methyl)-2-(N-benzyloxycarbonyl-L-leucinyl)hydrazine

Following the procedure of Example 1(e), except substituting 1-(N-*tert*-butoxycarbonyl)-1-(N-methyl)hydrazine for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and N-benzyloxycarbonyl-L-leucine for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a colorless oil (0.595 g, 85%). MS (ESI): 394.4 $(M+H)^+$.

c) 2-(N-benzyloxycarbonyl-L-leucinyl)-1-(N-methyl)hydrazine

Following the procedure of Example 7(b), except substituting 1-(N-*tert*-butoxycarbonyl)-1(-N-methyl)-2-(N-benzyloxycarbonyl-L-leucinyl)hydrazine for 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide, the title compound was prepared as a white solid (0.310 g, 70%). MS (ESI): 294.3 $(M+H)^+$.

d) 3-(2-pyridyl)phenylacetylhydrazide

Following the procedure of Example 1(b), except substituting methyl 3-(2-pyridyl)phenylacetate for methyl 3-benzyloxybenzoate, the title compound was prepared as an off-white solid (0.590 g, 100%). MS (ESI): 228.0 $(M+H)^+$.

e) 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1-(N-methyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 22(d), except substituting 2-(N-benzyloxycarbonyl-L-leucinyl)-1-(N-methyl)hydrazine for 1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine bis(hydrobromide) salt and 3-(2-pyridyl)phenylacetylhydrazide for N-benzyloxycarbonyl-L-leucinylhydrazide, the title compound was prepared as a white solid (0.034 g, 7%). MS (ESI): 548.3 $(M+H)^+$.

Example 30

Preparation of 2-[N-(N-picolinoyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-

benzyloxybenzoyl)]carbohydrazide and picolinic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.068 g, 50%). MS (ESI): 504.3 $(M+H)^+$.

Example 31

Preparation of 2-[N-[N-(2,6-dimethoxynicotinoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 2,6-dimethoxynicotinic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.086 g, 57%). MS (ESI): 564.2 $(M+H)^+$.

Example 32

Preparation of 2-[N-[N-(4-methanesulfonylbenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 4-methanesulfonylbenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.078 g, 50%). MS (ESI): 581.2 $(M+H)^+$.

Example 33

Preparation of 2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(3,4,5-trimethoxybenzoyl)-L-leucinyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-

benzyloxybenzoyl)]carbohydrazide and 3,4,5-trimethoxybenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.072 g, 45%). MS (ESI): 593.2 $(M+H)^+$.

Example 34

Preparation of 2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(8-quinolinoyl)-L-leucinyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and quinoline-8-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared was prepared as a white solid (0.075 g, 51%). MS (ESI): 554.3 $(M+H)^+$.

Example 35

Preparation of 2-[N-[N-[3,4-(1,3-propylenedioxy)benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 3,4-(1,3-propylenedioxy)benzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.091 g, 59%). MS (ESI): 575.2 $(M+H)^+$.

Example 36

Preparation of 2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-(N-thieno[2,3-b]thiophen-2-ylcarbonyl-L-leucinyl)]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-

benzyloxybenzoyl)]carbohydrazide and thieno[2,3-b]thiophene-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.072 g, 48%). MS (ESI): 565.0 $(M+H)^+$.

Example 37

Preparation of 2-[N-[N-(4-methoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 4-methoxybenzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.086 g, 60%). MS (ESI): 533.2 $(M+H)^+$.

Example 38

Preparation of 2-[N-[N-[4-methoxy-3-[2-(4-morpholino)ethoxy]benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) methyl 4-methoxy-3-(2-N-morpholinoethoxy)benzoate

Following the procedure of Example 1(a), except substituting methyl 3-hydroxy-4-methoxybenzoate for methyl 3-hydroxybenzoate and 4-(2-chloroethyl)morpholine hydrochloride for benzyl bromide, the title compound was prepared as a pale yellow oil (5.71 g, 65%). MS (ESI): 296.2 $(M+H)^+$.

b) 4-methoxy-3-[2-(4-morpholino)ethoxy]benzoic acid

Following the procedure of Example 14(c), except substituting methyl 4-methoxy-3-(2-N-morpholinoethoxy)benzoate for methyl 3-(2-pyridyl)phenylacetate, the title compound was prepared as a white solid (5.4 g, 100%). MS (ESI): 282.2 $(M+H)^+$.

c) 2-[N-[N-[4-methoxy-3-[2-(4-morpholino)ethoxy]benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 4-methoxy-3-[2-(4-morpholino)ethoxy]benzoic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.068 g, 38%). MS (ESI): 662.4 $(M+H)^+$.

Example 39

Preparation of 2-[N-[N-[2-(4-morpholino)pyrimidin-4-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) 2-methylthiopyrimidine-4-carboxylic acid potassium salt

To a suspension of 5-bromo-2-methylthiopyrimidine-4-carboxylic acid (1.25 g, 5.0 mmol) in methanol (60 mL) in a Parr bottle was added potassium hydroxide (0.630 g, 11.2 mmol) following by 10% palladium on $BaSO_4$ (0.630 g, 50% w/w). After shaking under hydrogen on a Parr shaker at 35psi for 3h, the mixture was filtered through Celite. The filtrate was concentrated to yield the title compound. ^1H NMR (400 MHz, CD_3OD) d 8.59 (d, 1H), 7.48 (d, 1H), 2.60 (s, 3H).

b) ethyl 2-methylthiopyrimidine-4-carboxylate

To a suspension of the compound of Example 39(a) in ethanol was added concentrated HCl (6 mL). After stirring at reflux for 16h, the solution was concentrated, dissolved in ethyl acetate, and washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated to yield the title compound as an oily yellow solid (0.851 g, 86%). ^1H NMR (400 MHz, $CDCl_3$) d 8.72 (d, 1H), 7.58 (d, 1H), 4.44 (q, 2H), 2.62 (s, 3H), 1.45 (t, 3H).

c) ethyl 2-methanesulfonylpyrimidine-4-carboxylate

To a solution of the compound of Example 39(b) (0.851g, 4.3mmol) in dichloromethane (50 mL) was added *m*-chloroperoxybenzoic acid (2.0 g, 11.6 mmol) slowly. After stirring at room temperature for 3h, the solution was diluted with

dichloromethane and washed with saturated aqueous sodium bicarbonate. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel; ethyl acetate/hexane) to yield the title compound as a white solid (0.788 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) d 9.16 (d, 1H), 8.17 (d, 1H), 4.50 (q, 2H), 3.43 (s, 3H), 1.45 (t, 3H).

d) ethyl 2-(4-morpholino)pyrimidine-4-carboxylate

The compound of Example 39(c) (0.200 g, 0.869 mmol) in morpholine (2 mL) was stirred at 80°C for 16h. The solution was diluted with ethyl acetate and washed with water and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel; ethyl acetate/hexane) to yield the title compound as a white solid (0.118 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) d 8.51 (d, 1H), 7.24 (d, 1H), 4.40 (q, 2H), 3.87 (t, 4H), 3.77 (t, 4H), 1.45 (t, 3H).

e) 2-(4-morpholino)pyrimidine-4-carboxylic acid

Following the procedure of Example 14(c), except substituting ethyl 2-N-morpholinopyrimidine-4-carboxylate for methyl 3-(2-pyridyl)phenylacetate, the title compound was prepared as a white solid (0.125 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) d 8.48 (d, 1H), 7.17 (d, 1H), 3.82 (t, 4H), 3.72 (t, 4H).

f) 2-[N-[N-[2-(4-morpholino)pyrimidin-4-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 2-(4-morpholino)pyrimidine-4-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.056 g, 44%). MS (ESI): 590.3 $(M+H)^+$.

Example 40

Preparation of 2-[N-[N-(2,3-dihydrobenzofuran-5-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 2,3-dihydrobenzofuran-5-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.070 g, 47%). MS (ESI): 545.4 $(M+H)^+$.

Example 41

Preparation of 2-[N-[N-(5-carboxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

a) 4-benzyloxycarbonylmethoxy-3-formylbenzyaldehyde

To a mixture of 5-formylsalicylaldehyde (2.2g, 14.7mmol) and potassium bromide (5.0 g, 36.8 mmol) in acetone (50 mL) was added benzyl bromoacetate (4.8 g, 16.1 mmol). After stirring at reflux for 6h, the mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated to yield the title compound (4.13 g, 94%). ^1H NMR (400 MHz, $CDCl_3$) d 10.56 (s, 1H), 9.95 (s, 1H), 8.38 (s, 1H), 8.07 (d, 1H), 7.38 (m, 5H), 6.95 (d, 1H), 5.26 (s, 2H), 4.91 (s, 2H).

b) benzyl 5-formylbenzofuran-2-carboxylate

Following the procedure of Example 20(c), except substituting 4-benzyloxycarbonylmethoxy-3-formylbenzyaldehyde for 4,5-dimethoxy-2-ethoxycarbonylmethoxybenzaldehyde, the title compound was prepared as a white solid (1.78 g, 46%). ^1H NMR (400 MHz, $CDCl_3$) d 10.09 (s, 1H), 8.24 (s, 1H), 8.05 (d, 1H), 7.71 (d, 1H), 7.68 (s, 1H), 7.42 (m, 5H), 5.43 (s, 2H).

c) benzyl 5-carboxybenzofuran-2-carboxylate

To a solution of the compound of Example 41(b) (0.380 g, 0.1.36 mmol) in THF (5 mL) and *tert*-butanol (1 mL) was added slowly a solution of sodium chlorite (0.245 g 2.71 mmol) and sulfamic acid (0.277 g, 2.86 mmol) in water (2 mL). After stirring at room temperature for 3h, the solution was partitioned between ethyl acetate and water. The organic layer was washed successively with water, saturated aqueous sodium bicarbonate, and brine then dried ($MgSO_4$), filtered and concentrated to yield the title compound as an off-white solid (0.272 g, 68%). ^1H NMR (400 MHz, $CDCl_3$) d 8.52 (s, 1H), 8.23 (d, 1H), 7.67 (m, 2H), 7.49 (m, 2H), 7.41 (m, 3H), 5.46 (s, 2H).

d) benzyl 5-*tert*-butoxycarbonylbenzofuran-2-carboxylate

To a solution of the compound of Example 41(c) (0.272 g, 0.919 mmol) in toluene (3 mL) was added N,N-dimethylformamide di-*tert*-butyl acetal (0.748 g, 3.68 mmol). After stirring at 80°C for 4h, the solution was concentrated and the residue dissolved in ethyl acetate. The organic layer was washed with water and brine then dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel, ethyl acetate/hexane) to yield the title compound as a white solid (0.144 g, 45%). ^1H NMR (400 MHz, $CDCl_3$) d 8.38 (s, 1H), 8.12 (d, 1H), 7.62 (m, 2H), 7.50 (m, 2H), 7.41 (m, 3H), 5.46 (s, 2H) 1.65 (s, 9H).

e) 5-*tert*-butoxycarbonylbenzofuran-2-carboxylic acid

Following the procedure of Example 20(a), except substituting benzyl 5-*tert*-butoxycarbonylbenzofuran-2-carboxylate for 2-benzyloxy-4,5-dimethoxybenzaldehyde, the title compound was prepared as a white solid (0.098 g, 91%). MS (ESI): 261.2 $(M-H)^-$.

f) 2-[N-[N-(5-*tert*-butoxycarbonylbenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and 5-*tert*-butoxycarbonylbenzofuran-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.123 g, 56%). MS (ESI): 643.2 $(M+H)^+$.

g) 2-[N-[N-(5-carboxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 7(b), except substituting 2-[N-[N-(5-*tert*-butoxycarbonylbenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide, the title compound was prepared as a white solid (0.160 g, 100%). MS (ESI): 587.2 $(M+H)^+$.

Example 42

Preparation of 2-[N-(3-benzyloxybenzoyl)-2'-[N'-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide

Following the procedure of Example 13(c), except substituting 2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(L-leucinyl)]carbohydrazide for 2,2'-[N,N'-bis-(L-leucinyl)]carbohydrazide bis(trifluoroacetate) salt, the title compound was prepared as an orange solid (0.012 g, 5%). MS (ESI): 674.2 $(M+H)^+$.

Example 43

Preparation of 2-[N-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]-2-[N-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 13(c), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide for 2,2'-[N,N'-bis-(L-leucinyl)]carbohydrazide bis(trifluoroacetate) salt, the title compound was prepared as an orange solid (0.038g, 19%). MS(ESI): 659.2 $(M+H)^+$.

Example 44

Preparation of 2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide

a) 3-(2-oxo-2-pyridinyl)phenylacetic acid

To a solution of the compound of Example 14(c) (1.2 g, 5.6 mmol) in diethyl ether (500 mL) and dichloromethane (10 mL) was added *m*-chloroperoxybenzoic acid (2.5 g, 8.5 mmol). After standing at room temperature for 36h, the white needles where collected and air dried for 2h to yield the title compound (1.1 g, 86%). MS (ESI): 230.1 $(M+H)^+$.

b) 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 5(a)-5(c), except substituting 3-(2-oxo-2-pyridinyl)phenylacetic acid for N-benzyloxycarbonyl-L-norvaline in step (c), the title compound was prepared as a white solid (0.842 g, 33%). MS (ESI): 549.2 $(M+H)^+$.

c) 2-(N-L-leucinyl)-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 15, except substituting 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide, the title compound was prepared as an off white solid (0.152 g, 100%). MS (ESI): 415.2 $(M+H)^+$.

d) 2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide

Following the procedure of Example 1(e), except substituting 2-(N-L-leucinyl)-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide for 2-[N-(3-benzyloxybenzoyl)]carbohydrazide and benzothiophene-2-carboxylic acid for N-benzyloxycarbonyl-L-valine, the title compound was prepared as a white solid (0.085 g, 47%). MS (ESI): 575.2 $(M+H)^+$.

Example 45

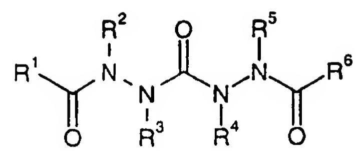
Preparation of 2,2'-[N,N'-bis[N-(3-iodo-4-methxoy)-L-leucinyl]]carbohydrazide

To a solution of the compound of Example 13(b) (0.200 g, 0.368 mmol), 3-iodo-4-methoxybenzoic acid (0.225 g, 0.809 mmol), triethylamine (0.082 g, 0.809 mmol), and 1-hydroxybenzotriazole (0.020 g, 0.147 mmol) in DMF (4 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.155g, 0.809mmol). After stirring at room temperature for 16h, the solution was diluted with ethyl acetate and washed successively with saturated aqueous sodium bicarbonate, water (2x) and brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (silica gel; methanol/dichloromethane) to yield the title compound as white solid (0.147 g, 48%). MS (ESI): 882.2 $(M+2Na)^+$.

The above specification and Examples fully disclose how to make and use the compounds of the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprise the state of the art and are incorporated herein by reference as though fully set forth.

We claim:

1. A compound of Formula I:



I

R^1 and R^6 are independently selected from the group consisting of C_{5-6}alkyl; C_{2-6}alkenyl; C_{3-11}cycloalkyl-C_{0-6}-alkyl; Ar-C_{0-6}alkyl; Het-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; Ar-C_{2-6}alkynyl; CH(R^7)Ar; CH(R^7)OAr; NR^7R^8; and CH(R^7)NR^8R^9;

R', R^2, R^3, R^4, R^5, R^8, R^{10}, R^{11}, and R^{14} are independently selected from the group consisting of H; C_{1-6}alkyl; C_{2-6}alkenyl; Ar-C_{0-6}alkyl; Het-C_{0-6}alkyl; and C_{3-11}cycloalkyl-C_{0-6}alkyl;

R^7 and R^{13} are independently selected from the group consisting of H; C_{1-6}alkyl; C_{2-6}alkenyl; C_{2-6}alkynyl; C_{3-11}cycloalkyl-C_{0-6}-alkyl; Ar-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Ar-C_{2-6}alkynyl; Het-C_{0-6}alkyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; C_{1-6}alkyl, which may optionally be substituted by OR^{10}, SR^{10}, and $NR^{10}R^{11}$; N(R')CO_2R'; CO_2R'; $CONR^{10}R^{11}$; and N(C=NH)NH_2;

R^7 and R^8 may optionally be combined to form a pyrrolidine or piperidine ring;

R^{10} and R^{11} may optionally be combined to form a pyrrolidine, piperidine, or morpholine ring;

R^9 is H; R^{12}; R^{12}C(O); R^{12}C(S); R^{12}OC(O); R^{12}OC(O)NR^{11}CH(R^{13})C(O); $R^{12}SO_2$; $R^{12}SO_2NR^{11}$CH(R^{13})C(O); R^{12}R'NC(O); R^{12}R'NCS; or COCH(R^{13})$NR^{14}R^{15}$;

R^{12} is C_{1-6}alkyl, which may be optionally substituted by $NR^{10}R^{11}$, C_{2-6}alkenyl, or C_{2-6}alkynyl; Ar-C_{0-6}alkyl; Ar-C_{2-6}alkenyl; Ar-C_{2-6}alkynyl; Het-C_{0-6}alkyl; Het-C_{2-6}alkenyl; Het-C_{2-6}alkynyl; C_{3-11}cycloalkyl, which may be optionally substituted with C_{1-6}alkyl, $(CH_2)_{1-6}CO_2$R', or adamantyl;

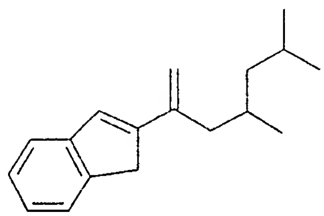
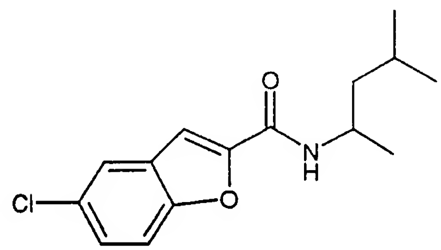
R^{15} is R^{12}; R^{12}C(O); R^{12}C(S); R^{12}OC(O); R^{12}OC(O)NR^{11}CH(R^{13})C(O); $R^{12}SO_2$,; $R^{12}SO_2NR^{11}$CH(R^{13})C(O); R^{12}R'NC(O); or R^{12}R'NCS;

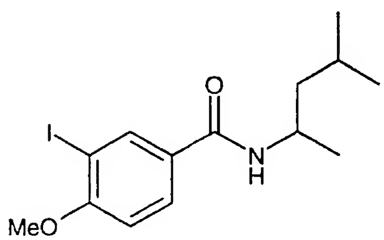
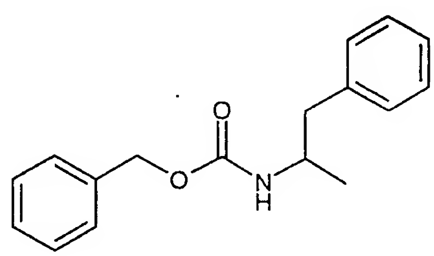
and pharmaceutically acceptable salts, hydrates and solvates thereof.

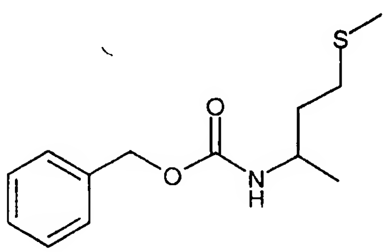
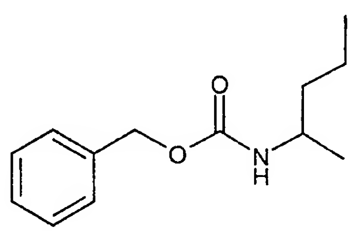
2. A compound according to Claim 1 wherein R^2, R^3, R^4, and R^5 are independently H.

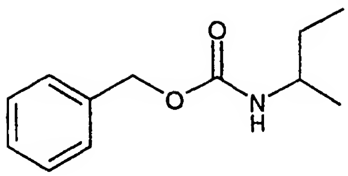
3. A compound according to Claim 1 wherein:

R^1 is independently:

 ;  ;

 ;  ;

 ;  ;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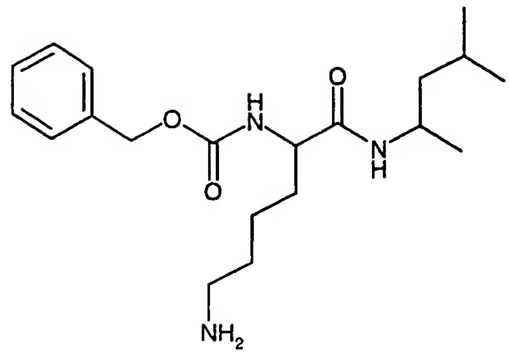
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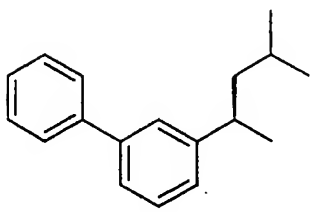
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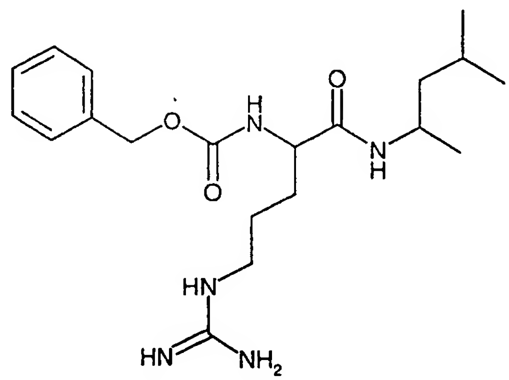
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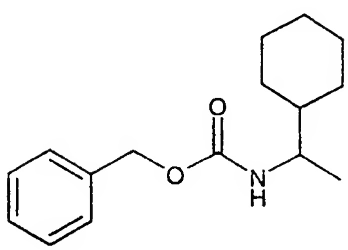
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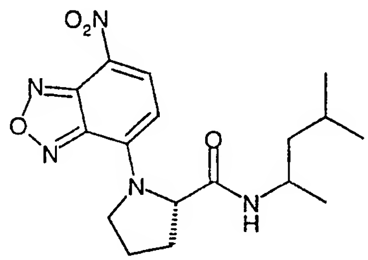
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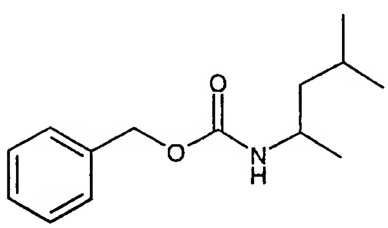
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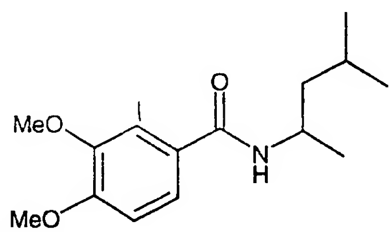
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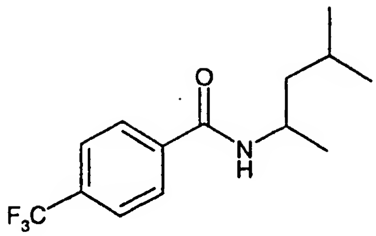
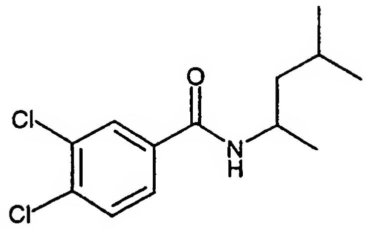
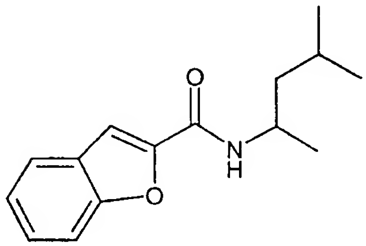
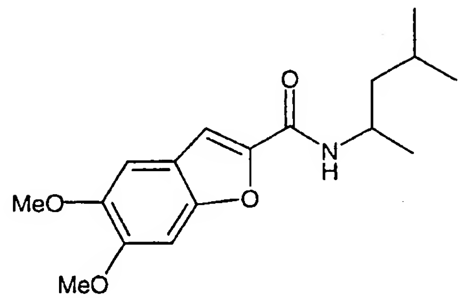
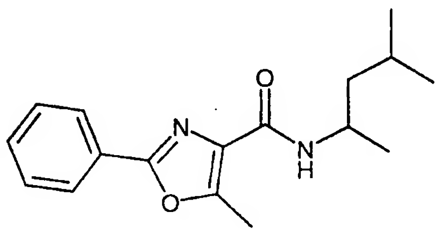
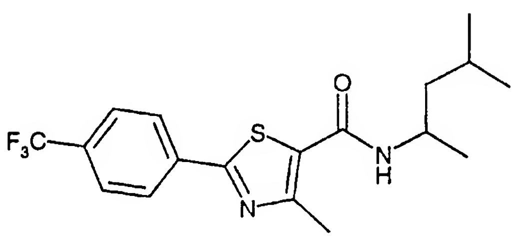
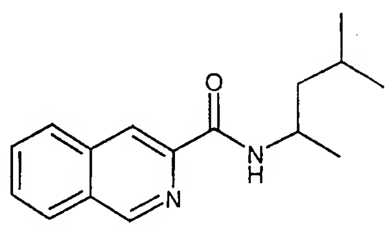
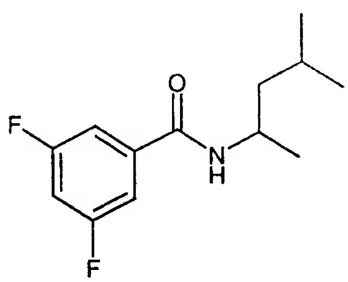
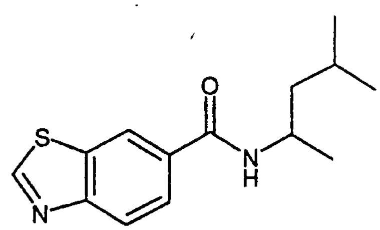
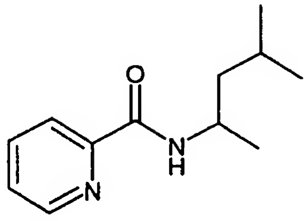
 ;  ;

 ;  ;

 ;

 ;  ;

 ;  ;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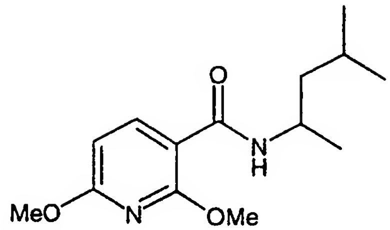
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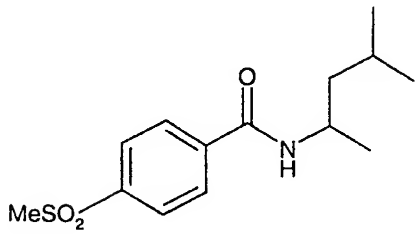
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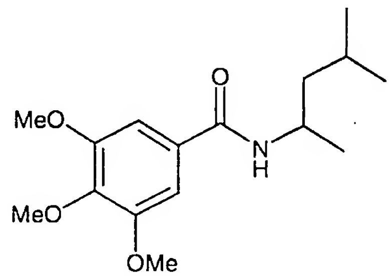
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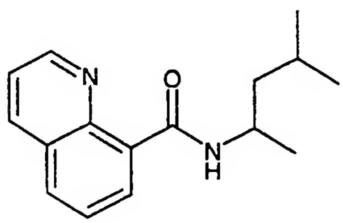
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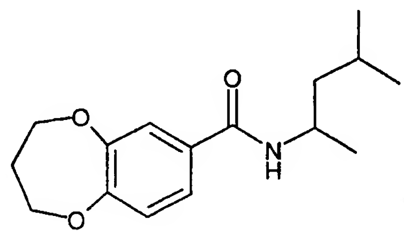
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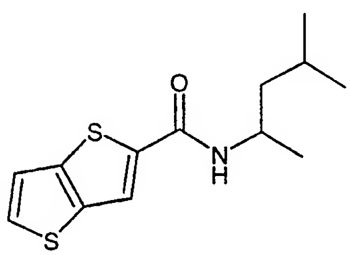
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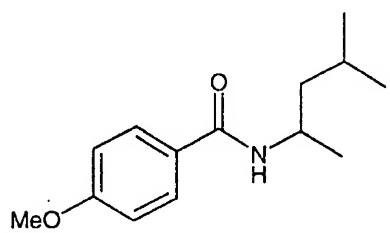
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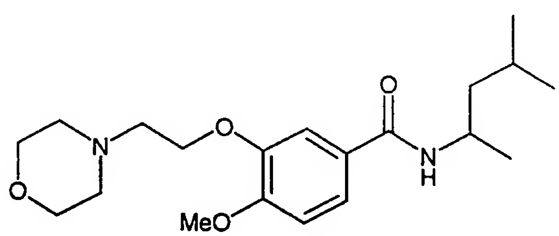
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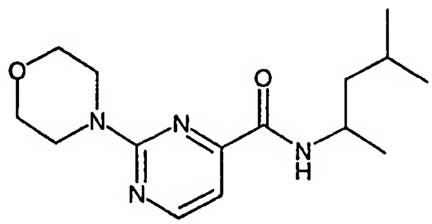

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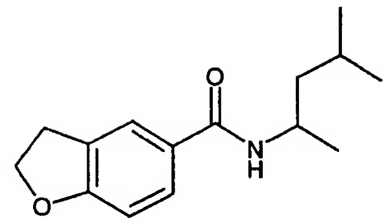

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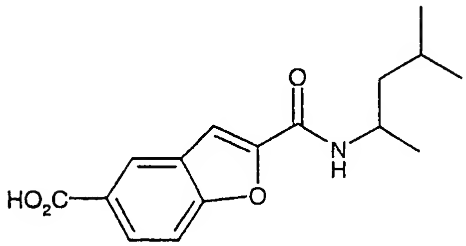

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;and

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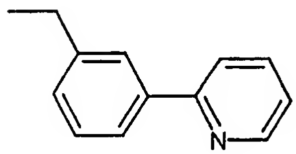
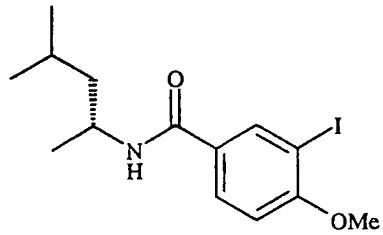
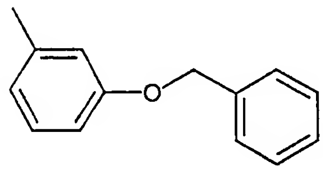
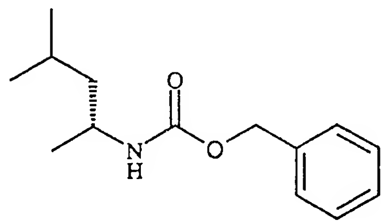
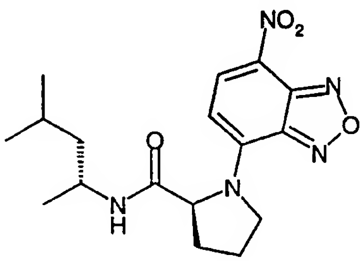
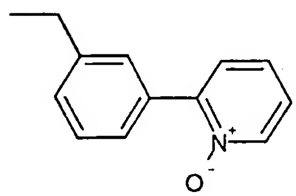
 ;

 ;

 ;

 ;

 ;



4. A compound of Claim 1 selected from the group consisting of:

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-valinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-phenylalanyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-isoleucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-methionyl)]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide;

(2S)-2-[N-(N-benzyloxycarbonyl-2-aminobutyryl)]-2'-[N'-(N-benzyloxycarbonyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide;

(2R)-2-[N-(3-benzyloxybenzoyl)]-2'-[N'-[4-methyl-2-(3-phenylphenyl)pentanoyl]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-arginyl-L-leucinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-norvalinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylglycinyl)]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-benzyloxycarbonyl-L-cyclohexylalanyl)]carbohydrazide;

2,2'-[N,N'-bis[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-(N-L-leucinyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3,4-dimethoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(4-trifluoromethylbenzoyl)-L-leucinyl]]carbohydrazide;

2-[N-[N-(3,4-dichlorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzofuran-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5,6-dimethoxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-methyl-2-phenyloxazol-4-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1'-N'-methyl-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3-isoquinolinoyl)-L-leucinyl]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-chlorobenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(3,5-difluorobenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzothiazol-6-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-1-(N-methyl)-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-picolinoyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(2,6-dimethoxynicotinoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(4-methanesulfonylbenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(3,4,5-trimethoxybenzoyl)-L-leucinyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-[N-(8-quinolinoyl)-L-leucinyl]]carbohydrazide;

2-[N-[N-[3,4-(1,3-propylenedioxy)benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[3-(2-pyridinyl)phenylacetyl]]-2'-[N'-(N-thieno[2,3-b]thiophen-2-ylcarbonyl-L-leucinyl)]carbohydrazide;

2-[N-[N-(4-methoxybenzoyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[4-methoxy-3-[2-(4-morpholino)ethoxy]benzoyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-[2-(4-morpholino)pyrimidin-4-ylcarbonyl]-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(2,3-dihydrobenzofuran-5-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-[N-(5-carboxybenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(3-benzyloxybenzoyl)-2'-[N'-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]carbohydrazide;

2-[N-[N-(3-nitro-1,2,7-benzoxadiazol-6-yl)-L-prolinyl-L-leucinyl]]-2-[N-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;
2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide; and
2,2'-[N,N'-bis[N-(3-iodo-4-methxoy)-L-leucinyl]]carbohydrazide.

5. A compound of Claim 4 selected from the group consisting of:
2-[N-(N-benzothiophen-2-ylcarbonyl-L-leucinyl)]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;
2-[N-[N-(5-chlorobenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide; and
2,2'-[N,N'-bis[N-(3-iodo-4-methxoy)-L-leucinyl]]carbohydrazide.

6. A pharmaceutical composition comprising a compound according to Claim 1 and a pharmaceutically acceptable carrier, diluent or excipient.

7. A pharmaceutical composition comprising a compound according to Claim 4 and a pharmaceutically acceptable carrier, diluent or excipient.

8. A method of inhibiting a protease selected from the group consisting of a cysteine protease and a serine protease, comprising administering to a patient in need thereof an effective amount of a compound according to Claim 1.

9. A method of inhibiting a protease selected from the group consisting of a cysteine protease and a serine protease, comprising administering to a patient in need thereof an effective amount of a compound according to Claim 4.

10. A method according to Claim 8 wherein said protease is a cysteine protease.

11. A method according to Claim 9 wherein said protease is a cysteine protease.

12. A method according to Claim 10 wherein said cysteine protease is cathepsin K.

13. A method according to Claim 11 wherein said cysteine protease is cathepsin K.

14. A method of treating a disease characterized by bone loss comprising inhibiting said bone loss by administering to a patient in need thereof an effective amount of a compound according to Claim 1.

15. A method according to Claim 14 wherein said disease is osteoporosis.

16. A method according to Claim 14 wherein said disease is periodontitis.

17. A method according to Claim 14 wherein said disease is gingivitis.

18. A method of treating a disease characterized by excessive cartilage or matrix degradation comprising inhibiting said excessive cartilage or matrix degradation by administering to a patient in need thereof an effective amount of a compound according to Claim 1.

19. A method according to Claim 18 wherein said disease is osteoarthritis.

20. A method according to Claim 18 wherein said disease is rheumatoid arthritis.

21. A method of treating a disease characterized by bone loss comprising inhibiting said bone loss by administering to a patient in need thereof an effective amount of a compound according to Claim 4.

22. A method according to Claim 21 wherein said disease is osteoporosis.

23. A method according to Claim 21 wherein said disease is periodontitis.

24. A method according to Claim 21 wherein said disease is gingivitis.

25. A method of treating a disease characterized by excessive cartilage or matrix degradation comprising inhibiting said excessive cartilage or matrix degradation by

administering to a patient in need thereof an effective amount of a compound according to Claim 4.

26. A method according to Claim 25 wherein said disease is osteoarthritis.

27. A method according to Claim 25 wherein said disease is rheumatoid arthritis.

28. A compound selected from the group consisting of:

2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide;
2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(L-leucinyl)]carbohydrazide;
2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_{α}-benzyloxycarbonyl-N_{ε}-*tert*-butoxycarbonyl-L-lysinyl-L-leucinyl)]carbohydrazide;
2-[N-(3-benzyloxybenzoyl)]-2'-[N'-(N_{α}-benzyloxycarbonyl-$N_{\phi 2}$-bis(*tert*-butoxycarbonyl)-L-lysinyl-L-leucinyl)]carbohydrazide;
2,2'-[N,N'-bis-(N-*tert*-butoxycarbonyl-L-leucinyl)]carbohydrazide;
2,2'-[N,N'-bis-(L-leucinyl)]carbohydrazide;
2-hydroxy-4,5-dimethoxybenzaldehyde;
4,5-dimethoxy-2-ethoxycarbonylmethoxybenzaldehyde;
ethyl 5,6-dimethoxybenzofuran-2-carboxylate;
5,6-dimethoxybenzofuran-2-carboxylic acid;
1-(N-benzyloxycarbonyl)-1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine;
1-(N-methyl)-2-[N-[3-(2-pyridyl)phenylacetyl]]hydrazine;
1-(N-tert-butoxycarbonyl)-1(-N-methyl)-2-(N-benzyloxycarbonyl-L-leucinyl)hydrazine;
2-(N-benzyloxycarbonyl-L-leucinyl)-1-(N-methyl)hydrazine;
3-(2-pyridyl)phenylacetylhydrazine;
ethyl 2-(4-morpholino)pyrimidine-4-carboxylate;
2-(4-morpholino)pyrimidine-4-carboxylic acid;
4-benzyloxycarbonylmethoxy-3-formylbenzyaldehyde;
benzyl 5-formylbenzofuran-2-carboxylate;
benzyl 5-carboxybenzofuran-2-carboxylate;
benzyl 5-*tert*-butoxycarbonylbenzofuran-2-carboxylate;
5-*tert*-butoxycarbonylbenzofuran-2-carboxylic acid;

2-[N-[N-(5-*tert*-butoxycarbonylbenzofuran-2-ylcarbonyl)-L-leucinyl]]-2'-[N'-[3-(2-pyridinyl)phenylacetyl]]carbohydrazide;

2-[N-(N-benzyloxycarbonyl-L-leucinyl)]-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide; and

2-(N-L-leucinyl)-2'-[N'-[3-(2-oxo-2-pyridinyl)phenylacetyl]]carbohydrazide.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17275

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/237.8, 307, 357, 364, 367, 374, 443, 469, 590; 544/168; 546/146, 332; 548/125, 180, 236; 549/50, 441; 564/34, 37

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN/CAS, structure search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,038,247 A (MULLER et al.) 26 July 1977, whole document.	1-7, 28
X	WO 97/16433 A (SMITHKLINE BEECHAM CORPORATION) 09 May 1997, whole document.	1-28
X	Chem. abstr. Vol. 83, 1971 (Columbus, OH), DN 83: 180329, KAWADA et al., 'Polymer Compositions', JP 50058142, 20 May 1975.	1-7, 28
X	THOMPSON et al. Design of Potent and Selective Human Cathepsin K Inhibitors that Span the Active Site. Proc. Natl. Acad. Sci. December 1997, Vol. 94, pages 14249-14254.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 NOVEMBER 1998

Date of mailing of the international search report
29 DEC 1998

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RICHARD L. RAYMOND
D. Lawrence for
Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17275

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 31/175, 31/34, 31/38, 31/41, 31/42, 31/425, 31/44, 31/47. 31/535; C07C 281/06; C07D 213/54, 217/16, 263/32, 265/30, 277/64, 307/81, 413/04, 495/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/237.8, 307, 357, 364, 367, 374, 443, 469, 590; 544/168; 546/146, 332; 548/125, 180, 236; 549/50, 441; 564/34, 37